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FUSARIUM DISEASES OF BROAD BEAN. III. ROOT-ROT AND WILT OF BROAD BEANS CAUSED BY TWO NEW FORMS OF FUSARIUM[1]

T. F. Yu and C. T. Fang[2]

(Accepted for publication January 10, 1948)

INTRODUCTION

Foot-rot, root-rot, and wilt are very destructive diseases in the broad-bean growing regions of Yunnan, China, especially where proper rotation has not been practiced. They have been found in all regions where beans are grown extensively and in certain sections have caused annual losses as high as 40 per cent of the crop.

The causes of these diseases were found to be species of *Fusarium*. One of them is *Fusarium avenaceum* var. *fabae* which causes a wilt disease. The results of an investigation of the morphology and physiology of this fungus together with the disease induced by it have been reported in preceding papers (8, 9). In the present paper, two additional species of *Fusarium* parasitizing broad bean are described.

THE ROOT-ROT FUNGUS

Cultural Characters of Fusarium solani f. fabae n.f.

Hard potato agar. Cultures 1 month old are characterized by a loose, coarse, wooly, granular, white to cartridge buff mycelium (4, 5). Pionnotes, produced abundantly in large heaps, are putty buff and cream buff. Older cultures have thick and more or less compact mycelial growth that is laelia pink, Burgundy purple, and Rigi blue. Sclerotia are small, Rigi blue to slate color, and usually imbedded in the mycelia. Numerous India buff sporodochia are produced.

Hard oat agar. Cultures 1 month old are characterized by a medium coarse to fine wooly mycelium that is white to buff. Sporodochia and pionnotes are produced in large groups over the slant. Sclerotia are produced abundantly in masses and are spruce green to Rigi blue.

Broad-bean seed-decoction agar. Mycelium is scant, white, and cottony on 12-day-old cultures; scant, white, and wooly on one-month-old cultures. Small putty-buff sporodochia are generally formed on the basal portion of the slant. Sclerotia are not seen.

Potato-tuber plug. Cultures 1 month old have a loose, coarse, wooly, granular mycelium that is white, light Martius yellow, and Rigi blue. Large masses of sporodochia that run together, forming pionnotes, are usually produced. They are usually cinnamon to powdered yellow. The abundant sclerotia are blue spruce green to Rigi blue.

[1] Paper No. 34 from Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University.

[2] Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University.

Melilotus stem. One-month-old growth is characterized by a loose, coarse, wooly, granular, white to buff mycelium. Cinnamon to powdered yellow pionnotes and sporodochia are present over the stem in small and large heaps. Small, Rigi blue sclerotia may be present.

Green broad bean pod. Cultures 2 months old are characterized by a medium thick, matted, cartridge buff and ivory buff mycelium. Large, spherical cinnamon sporodochia are produced. Sclerotia are small and Rigi blue.

Broad bean stem. Cultures 1 month old have scant, white to cartridge buff aerial mycelium. Small, pale pinkish buff sporodochia are produced.

Rice. Cultures 12 days old have a moderately compact, matted mycelium that is white, cartridge buff and rosewood red, while the rice is changed to coral pink. Small, Rigi blue sclerotia are developed. Cultures 1 month old are characterized by scant, white, aerial mycelium. The rice is rosewood red to prune purple and Rigi blue to slate color. Sporodochia are putty buff to sheepskin moth yellow, single or aggregated, and circular or irregular in outline.

Potato agar plate, 5 per cent dextrose. Cultures 1 month old have a white to cartridge buff aerial mycelium, and that in the substratum is cinnamon buff and rosewood red. The cultures are zonate. Sporodochia may be present in the center of the plate. Microconidia are produced in great abundance.

Measurements of Conidia from Sporodochia Produced on Different Media

Hard oatmeal agar; cultures 44 days old:

3-septate, 57 per cent, 38.8×5.2 ($31.8–44.1 \times 4.8–5.4$) μ.
4-septate, 21 per cent, 40.9×5.2 ($35.3–49.4 \times 4.9–5.3$) μ.
5-septate, 22 per cent, 42.7×5.2 ($38.8–47.6 \times 3.9–5.3$) μ.

Cornmeal agar; cultures 44 days old:

3-septate, 78 per cent, 34.4×5.4 ($23.6–40.0 \times 3.6–5.5$) μ.
4-septate, 13 per cent, 40.3×5.5 ($32.7–50.9 \times 5.3–5.8$) μ.
5-septate, 9 per cent, 43.3×5.6 ($38.2–47.3 \times 5.5–6.2$) μ.

Diseased broad bean plants; plants killed two weeks after inoculation:

0-septate, 1 per cent, 19.4×3.0 μ.
1-septate, 2 per cent, 21.7×3.4 ($21.2–22.9 \times 3.2–3.7$) μ.
2-septate, 2 per cent, 32.6×4.8 ($31.8–33.5 \times 4.4–5.3$) μ.
3-septate, 82 per cent, 32.9×4.7 ($26.5–42.4 \times 3.5–5.3$) μ.
4-septate, 7 per cent, 36.7×5.0 ($31.9– 4.2 \times 3.5–5.3$) μ.
5-septate, 6 per cent, 38.6×4.9 ($35.3–42.4 \times 3.9–5.3$) μ.

Hard potato agar, 2 per cent dextrose; cultures 44 days old:

1-septate, 2 per cent, 20.0×3.3 μ.
2-septate, 4 per cent, 21.8×3.7 ($20.0–23.6 \times 3.6–3.8$) μ.
3-septate, 78 per cent, 32.4×5.5 ($25.5–40.0 \times 4.0–5.8$) μ.
4-septate, 12 per cent, 35.8×5.5 ($32.7–38.2 \times 5.5–5.6$) μ.
5-septate, 4 per cent, 38.2×5.5 ($41.5–48.5 \times 5.1–5.6$) μ.

Broad bean seed-decoction agar; cultures 138 days old:

3-septate, 13 per cent, 30.6×5.5 ($26.5–34.4 \times 5.3–6.2$) μ.
4-septate, 22 per cent, 35.6×5.5 ($31.8–42.4 \times 5.3–6.5$) μ.
5-septate, 62 per cent, 40.5×5.6 ($32.6–47.6 \times 5.1–7.1$) μ.
6-septate, 3 per cent, 44.6×5.5 ($41.5–48.5 \times 5.1–5.6$) μ.

Potato-tuber plug; cultures 30 days old:

3-septate, 82 per cent, 33.7×5.3 ($26.5–45.9 \times 4.1–5.9$) μ.
4-septate, 12 per cent, 36.0×5.2 ($32.6–40.6 \times 4.8–5.3$) μ.
5-septate, 6 per cent, 38.8×5.3 ($35.3–42.4 \times 5.1–5.4$) μ.

Melilotus stem; cultures 44 days old:

3-septate, 37 per cent, 34.9×5.3 ($23.6–41.8 \times 4.2–5.6$) μ.
4-septate, 44 per cent, 38.1×5.4 ($32.7–45.5 \times 5.1–5.5$) μ.
5-septate, 19 per cent, 40.2×5.4 ($36.4–43.6 \times 4.7–5.5$) μ.

Green broad bean pod; cultures 138 days old:

3-septate, 36 per cent, 33.5×5.1 ($27.4–37.1 \times 3.9–5.3$) μ.
4-septate, 30 per cent, 36.5×5.2 ($32.6–42.4 \times 4.8–5.6$) μ.
5-septate, 33 per cent, 37.8×5.6 ($32.6–42.4 \times 4.6–5.3$) μ.
6-septate, 1 per cent, 38.8×5.3 μ.

Green pea pod; cultures 138 days old:

3-septate, 55 per cent, 32.5×5.1 ($28.2–40.6 \times 3.9–5.6$) μ.
4-septate, 23 per cent, 33.7×5.2 ($27.3–37.1 \times 3.9–5.6$) μ.
5-septate, 22 per cent, 38.8×5.3 ($33.6–40.6 \times 3.9–5.6$) μ.

Robinia root; cultures 32 days old:

3-septate, 44 per cent, 34.1×5.3 ($26.5–38.1 \times 4.1–6.0$) μ.
4-septate, 30 per cent, 37.0×5.3 ($31.8–42.4 \times 4.6–5.6$) μ.
5-septate, 26 per cent, 38.8×5.3 ($31.8–42.4 \times 4.9–6.2$) μ.

Steamed rice; cultures 44 days old:

3-septate, 44 per cent, 39.0×5.2 ($26.5–46.8 \times 4.1–5.2$) μ.
4-septate, 19 per cent, 41.6×5.2 ($31.8–46.8 \times 4.1–5.5$) μ.
5-septate, 37 per cent, 43.2×5.3 ($35.5–52.8 \times 4.1–5.4$) μ.

The averages for conidia on different media of various ages are as follows:

0-septate, 19.4×3.0 μ.
1-septate, 20.8×3.3 ($20.0–21.7 \times 3.3–3.4$) μ.
2-septate, 27.2×4.2 ($21.8–32.6 \times 3.7–4.8$) μ.
3-septate, 34.8×5.2 ($30.6–39.0 \times 4.8–5.5$) μ.
4-septate, 38.3×5.2 ($35.5–41.6 \times 4.9–5.5$) μ.
5-septate, 40.2×5.3 ($38.2–43.3 \times 4.9–5.6$) μ.
6-septate, 44.9×5.3 ($38.8–53.4 \times 5.0–5.5$) μ.

Morphology of the Fungus

Microconidia borne on irregularly branched conidiophores, ovoid, oblong, or short rod-shaped, 6.6×2.1 μ, rarely 1-septate, 12.8×2.6 μ; macroconidia scattered in mycelium or in false heads, in sporodochia or pionnotes, spindle-shaped or slightly curved, with rounded to slightly constricted apex, slightly or not at all pedicellate, 0- to 6-septate, typically 3-septate, 34.8×5.2 μ; conidial mass gold yellow, putty buff, cinnamon or cream colored; sclerotia small, Rigi blue or slate color; chlamydospores terminal or intercalary, mostly 1-celled, spherical or oblong, 10.6×10.0 μ, 2-celled, 24.2×15.1 μ, sometimes in short chains, smooth or sometimes rugose (Fig. 1, A, B, and C).

This fungus, isolated from diseased broad bean root and proven to be parasitic on the host, is practically identical with *Fusarium solani* (Mart.) App. et Wr. (7), especially in spore measurements and cultural character-

istics. The number of septa in conidia and the proportion of conidia having the same number, also are similar. *Fusarium solani* generally is regarded as a saprophyte and only under exceptional circumstances may it become a weak wound-parasite. The broad bean fungus is undoubtedly parasitic and highly virulent. There are two other species of *Fusarium* closely related to the present fungus and able to cause root-rot on legumes. One of them is *Fusarium solani* v. *martii* forma 2 (*Fusarium martii* v. *pisi*) the causal fungus of root-rot of peas (2, 7); and the other is F. *solani* v. *martii* forma 3 (*Fusarium martii-phaseoli*) the causal fungus of root-rot of beans (2, 7). The present fungus differs from them in spore size and in the host attacked. Since pathogenicity has been used in the genus *Fusarium* as the basis for distinguishing new forms, it seems justifiable to consider the present fungus as a new form of *F. solani* and the trinomial *F. solani* f. **fabae** is proposed.

Pathogenicity

This fungus causes the typical root-rot disease on broad bean. The infected roots and basal portions of the stems become blackened and rotted. At the late stage of development of the disease, the lateral roots and the greater portion of tap roots shrivel and become dry. The leaves are only slightly yellow. The most characteristic symptom of the disease of the aerial portion of the plant is the production of black lesions of varying size on the margins of the lower leaves. These lesions spread gradually over the surface until the whole leaf is involved. On the upper leaves, black spots, irregular in shape and varied in size, appear between the veins. As the disease progresses, the leaves and stems become blackened, shrivelled, and dead. Both the cortex and vascular tissues of the root and basal portion of the stem are invaded by the fungus and become brown.

The results of cross inoculation experiments indicate that this fungus is unable to cause infection on beans, peas, cowpeas, oats, wheat, and corn.

THE WILT FUNGUS

Cultural Characters of Fusarium oxysporum f. fabae n.f.

Hard potato agar. Cultures 14 days old are characterized by a fine, thick, matted mycelium that is dirty white. Old cultures, between 1 and 3 months old, are the same but with additional blue or blue-green pigmentation as a narrow lip where the lower edge of the slant surface touches the glass.

Hard, old agar. Cultures 22 days old have a thick, compact, dense, matted aerial mycelium that is dirty white and light Highland green, especially on the sides at the base of the slant. Sclerotial stromata are Highland green.

Broad bean seed-decoction agar. Cultures 5 months old have scant, loose, and white mycelial growth that may have blue-green pigmentation.

Potato-tuber plug. Cultures 22 days old have a medium dense, leathery, dirty white mycelium, sometimes with spots of sheepskin moth yellow, slate color, dark chessylite and blue-green in places. Sclerotial stromata are not present.

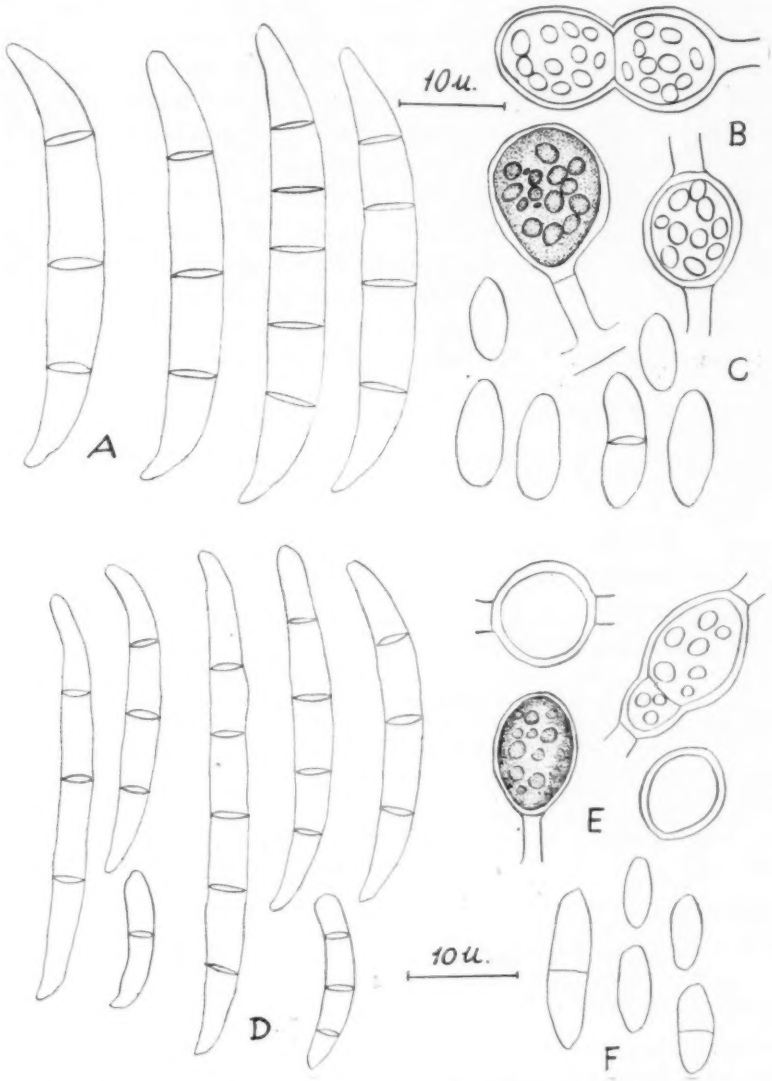

Fig. 1. *Fusarium solani* f. *fabae:* A. Macroconidia; B. Chlamydospores; C. Microconidia. *Fusarium oxysporum* f. *fabae:* D. Macroconidia; E. Chlamydospores; F. Microconidia.

Melilotus stem. Cultures 1 month old have a medium scant, white, tawny, and Arnoblue mycelium with occasional tufts that are white and wooly. Sclerotial stromata may be present.

Green broad bean pod. Cultures 5 months old have a medium compact

mycelium that is dirty white and Rigi blue. The mycelium may be slimy or self-digested.

Green pea pod. The cultural characters of the fungus on pea pod are the same as on broad bean pod except that in a few instances small brown sporodochia may be produced. Sclerotial stromata are not present.

Rice. Cultures 12 days old have a medium dense, dirty white mycelium. Older cultures, from 1 to 2 months old, are characterized by a leathery mycelium that is La Valliere to dahlia purple, Crown Prince gray, dusky blue, and slate color. There are also spots of cream-buff, yellow ocher, coral pink, and Isabella color.

This fungus produces relatively abundant microconidia and sometimes sclerotial stromata on these cultural media. However, macroconidia are rarely produced, and sporodochia or pionnotes are often absent. The mycelial growth is at first dirty white, compact, and dense. In most of the tube cultures the presence of a greenish blue narrow lip on the lower edge of the slant surface is very characteristic.

Measurements of Conidia on Different Media

Diseased broad bean plants; conidia from aerial mycelium:

1-septate, 3 per cent, 23.2×3.5 ($22.0–23.8 \times 3.4–3.9$) μ.
2-septate, 3 per cent, 26.1×3.6 ($26.7–27.3 \times 3.5–3.9$) μ.
3-septate, 91 per cent, 35.8×3.6 ($25.6–44.1 \times 3.2–5.3$) μ.
4-septate, 2 per cent, 37.9×4.1 ($35.3–40.6 \times 3.7–4.4$) μ.
5-septate, 1 per cent, 47.6×4.9 μ.

Diseased broad bean plants in moist chamber; cultures 30 days old; conidia from aerial mycelium:

1-septate, 29 per cent, 21.0×3.5 ($14.1–26.4 \times 3.2–4.4$) μ.
2-septate, 11 per cent, 26.8×3.6 ($24.6–29.9 \times 3.3–3.9$) μ.
3-septate, 54 per cent, 32.8×4.0 ($22.9–51.0 \times 3.5–5.3$) μ.
4-septate, 6 per cent, 39.9×4.6 ($35.2–46.2 \times 3.5–5.3$) μ.

Pea pod; cultures 74 days old; conidia from sporodochia:

3-septate, 90 per cent, 29.2×3.8 ($21.8–36.4 \times 3.5–4.9$) μ.
4-septate, 8 per cent, 35.1×3.7 ($23.6–34.5 \times 3.5–3.8$) μ.
5-septate, 2 per cent, 37.3×4.0 ($36.4–38.2 \times 3.8–4.2$) μ.

Melilotus stem; cultures 48 days old; conidia from aerial mycelium:

1-septate, 4 per cent, 23.2×3.3 ($15.4–26.1 \times 3.2–4.4$) μ.
2-septate, 4 per cent, 25.5×3.3 ($24.6–27.8 \times 3.2–4.3$) μ.
3-septate, 88 per cent, 35.1×4.0 ($29.9–47.5 \times 3.5–4.9$) μ.
4-septate, 4 per cent, 43.0×4.6 ($39.8–48.1 \times 3.5–4.9$) μ.

Robinia root; cultures 74 days old; conidia from aerial mycelium:

1-septate, 1 per cent, 15.0×2.1 μ.
2-septate, 1 per cent, 15.9×3.0 μ.
3-septate, 91 per cent, 24.7×3.3 ($17.6–37.1 \times 2.6–3.9$) μ.
4-septate, 4 per cent, 33.5×3.4 ($26.5–37.1 \times 3.2–3.5$) μ.
5-septate, 3 per cent, 33.7×3.5 ($31.8–38.8 \times 3.2–3.7$) μ.

The averages of these measurements of conidia on different media of various ages are as follows:

0-septate, 8.9×2.9 μ.
1-septate, 17.8×3.0 ($9.9–23.2 \times 2.1–3.5$) μ.

2-septate, 25.7×3.5 ($15.9–26.8 \times 3.0–3.6$) μ.
3-septate, 31.9×3.7 ($24.7–35.8 \times 3.3–4.0$) μ.
4-septate, 38.0×4.1 ($33.5–43.0 \times 3.4–4.6$) μ.
5-septate, 40.6×4.1 ($33.7–47.6 \times 3.5–4.9$) μ.

Morphology of the Fungus

Mycelium vigorous, dirty white at first, becoming tawny to variously tinted, pigment of substratum highly variable; microconidia formed free in aerial mycelium, relatively abundant, ovoid, oblong or irregularly ellipsoid, mostly 0-septate, 7.1×3.3 ($5.2–10.4 \times 2.1–3.5$) μ; macroconidia rarely produced, mostly 3-septate, nearly uniform in diameter or slightly broader toward the upper end, slightly curved at the upper end, usually with rounded apex and with base nearly straight-conical or appendicular, seldom subpedicellate, 31.9×4.1 μ; sporodochia and pionnotes rarely produced; sclerotial stromata present, in certain cultural media, Highland green and spruce green to marble green; chlamydospores single, in pairs, or in short chains, terminal or intercalary, spherical to subspherical, thick-walled, dark brown, smooth, 7.3×6.9 ($6.6–9.1 \times 5.8–7.4$) μ (Fig. 1, D, E, and F).

The foregoing description of the fungus causing wilt of broad bean shows that it resembles *Fusarium oxysporum* emended by Snyder and Hansen (6) and it especially resembles *F. oxysporum* f. *pisi* (3, 7) in both cultural and morphologic characteristics. It differs, however, from the pea fungus in pathogenicity. Cross inoculation experiments were made with this fungus and two cultures of *F. oxysporum* f. *pisi*. One of these pea fungus cultures was received through the kindness of Dr. J. C. Walker of United States and the other was isolated by the writers from peas in Yunnan. The results of these experiments indicate the pea fungi occasionally cause infection on broad bean. On the other hand, it has been impossible so far to cause infection on peas with the broad bean fungus. Following the system of classification of *F. oxysporum* proposed by Snyder and Hansen (6), the present fungus will be named *F. oxysporum* f. **fabae** as a new form of this species.

Pathogenicity

The most characteristic symptom of the disease caused by this fungus on the aerial portion of broad beans is the yellowing of the leaves. Affected leaves become yellow and more or less rigid. The roots and basal portion of the stems of a diseased plant are not conspicuously discolored. When the diseased roots are cut open, the vascular tissues have the characteristic reddish brown discoloration.

Inoculations of beans, cowpeas, peas, oats, wheat, and corn with this fungus were negative.

RÉSUMÉ

Two new forms of *Fusarium* that cause diseases in *Vicia faba* L. are reported in this paper. One of them, causing the root-rot of broad bean,

is named *F. solani* f. *fabae* and the other, causing wilt of the same host, is named *F. oxysporum* f. *fabae*. Their cultural characteristics, morphology, and pathogenicity are described.

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THE DEVELOPMENT OF GIBBERELLA ZEAE HEADBLIGHT OF WHEAT

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INTRODUCTION

Although wheat headblight or scab caused by *Gibberella zeae* (Schw.) Petch has been investigated frequently during the past 60 years, some aspects of the disease still need clarification. The literature has been reviewed adequately by Atanasoff (2), MacInnes and Fogelman (9) and Eide (6).

This paper includes studies on the effects of temperature on growth, sporulation, and spore germination of the pathogen; the effects of the inoculum dosage, stage of host maturity, and air temperature and air moisture on disease development.

MATERIALS AND METHODS

Laboratory Studies

A single monoconidial isolate of *Gibberella zeae* was used throughout the investigations. This culture was selected from several obtained from Dr. J. J. Christensen of the University of Minnesota, because of its pathogenicity on seedling wheat.

The conidial stage of *Gibberella zeae* (*Fusarium graminearum* Schwabe) was produced on Coons' agar initially adjusted to pH 3.2 using normal HCl. This medium was used in the temperature studies on growth and sporulation and also for producing the conidia used for plant inoculation.

The effect of temperature on growth, sporulation, and germination was studied in incubators maintained within $\pm$ 0.5° C. of the desired temperature. Growth rates were determined on agar in Petri plates which had been seeded with a 5-mm. agar plug in the center of the plate. Diameter of the mycelia was recorded after 5 days. Methods similar to those presented by Henry and Andersen (7) were used in the sporulation studies.

Spore germination was studied by placing 2 to 4 drops of a dilute conidial suspension of *Gibberella zeae* on a clean glass slide. The slide was placed in a Petri plate during the incubation period to prevent the water from evaporating. At least 200 germinated and non-germinated conidia were counted on each slide and the percentage germination determined on the basis of these counts.

Greenhouse Studies

Newthatch spring wheat was the principal variety used in the greenhouse experiments. The wheat was sown in soil in one-gallon glazed pots and thinned to 10 plants per pot shortly after emergence. It was grown at relatively cool temperatures (12° to 15° C.) for the first two months,

and thereafter in a warm greenhouse (20° to 25° C.) with supplementary lighting. Uniform heads were obtained nearly identical in appearance to those produced on wheat grown in the field.

Plants were inoculated at five stages of development as follows: (1) the boot stage, in which the head was still enclosed in the leaf sheath; (2) the before-flowering stage, in which the heads were out of the boot but none of the flowers had extruded anthers; (3) the flowering stage, in which the flowers were in the process of anthesis or had already extruded anthers; (4) the after-flowering stage, in which small immature seeds were present and (5) the past-flowering stage in which the kernels were nearly filled out and in the milk to soft dough stage.

The technique used for plant inoculation was the same as that reported by Andersen *et al.* (1). This consisted in the application of a suspension of conidia by means of a hand-made atomizer. Unless mentioned otherwise, the standard application of inoculum in all experiments was approximately two million conidia per pot. Conidial germination was checked prior to the use of the inoculum to insure the application of viable conidia.

In all the studies on pathogenesis, the plants were exposed to continued wetness for different periods in the humidity tents before they were removed to the greenhouse bench to complete their incubation period. Greenhouses were maintained at approximately 25° C.

RELATION OF TEMPERATURE TO GROWTH, SPORULATION, AND SPORE GERMINATION IN VITRO

The rate of growth of *Gibberella zeae* on Coons' agar was determined after five days at different temperatures. There was a very rapid increase in the rate of growth from 8° to 28° C. with an especially significant increase between 20° and 24° C. The growth rate at 32° C. was comparable to that obtained at 12° C. and no growth resulted at 36° C. Slow growth occurred at 4° C., so the minimum temperature is probably slightly below 4° C.

Studies on the effect of temperature on spore production were conducted at the same temperatures as in the growth rate studies. The most rapid spore production occurred initially at 28° and 32° C., but on longer incubation (after 7 and 12 days) considerably more conidia were produced at 32° C. (Fig. 1, A). The strain used in these studies produced very few conidia at temperatures below 20° C. and none at 36° C. Thus the best temperature for sporulation in culture was not the same as that for growth.

Microscopic observations made while making spore counts indicated that spores from cultures incubated at different temperatures differed morphologically. In order to determine whether the incubation temperatures exerted an influence on the length of the conidia, measurements and records of 20 to 25 conidia from one culture incubated at each of the temperatures favoring good sporulation (20° to 32° C.) were taken at random. At 20° and 24° C., 60 to 70 per cent of the conidia were 5-septate and the remain-

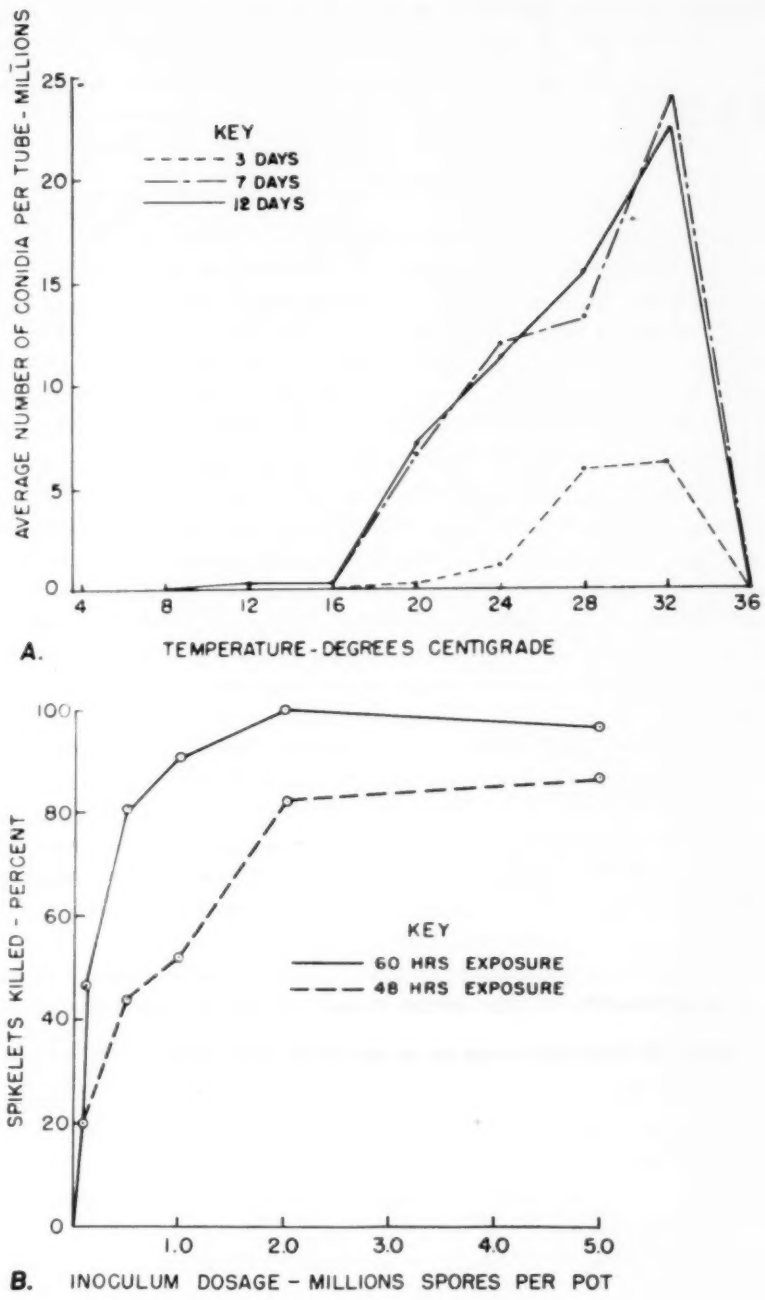

FIG. 1. A. Effect of temperature on sporulation by *Gibberella zeae* on Coons' agar. B. The effect of inoculum dosage on the development of Gibberella headblight on wheat inoculated in the flowering stage.

TABLE 1.—*Effect of temperature on the germination of Gibberella zeae conidia*

Temperature, degrees C.	Time in hours			
	6	24	48	72
	Percentage germination[a]			
4	0.0	0.0	88.9	93.7
8	0.0	17.4	95.6	.....
12	0.0	85.9	96.8	.....
16	50.3	88.5	.....	.....
20	85.2	.....	.....	.....
24	89.7	.....	.....	.....
28	93.5	.....	.....	.....
32	91.0	.....	.....	.....
37	0.0	0.0	0.0	0.0

[a] Based upon counts of 200–400 conidia for each sample.

der 3- and 4-septate. At 28° C. only 27 per cent were 5-septate while over 40 per cent were 3-septate and the remainder 4-septate. At 32° C., over 50 per cent were 2-septate, more than 30 per cent were 3-septate, and a few were 1- and 4-septate. Conidia with a like number of septations were approximately equal in length at all temperatures. For example, the 3-septate conidia measured 37.5–41.2 μ; the 4-septate 41.0–44.1 μ; and the 5-septate 47.9–49.5 μ. Thus temperature influenced the length of the conidia produced on artificial media through a reduction in the number of septa in each conidium.

The conidia of *Gibberella zeae* germinate very rapidly. Many were observed to have germinated in 3 hours at 28° or 32° C. In 6 hours, nearly all the conidia exposed at 20°, 24°, 28°, and 32° C. had germinated (Table 1). At lower temperatures the rate of germination was considerably slower, although the percentage germination at the lower temperatures after 48 and 72 hours of incubation was as high as that recorded at 28° C. in 6 hours.

Records on the percentage germination of the conidia (Table 1) supplemented by measurements of the germ tubes (Table 2) gave more reliable

TABLE 2.—*Effect of temperature on the length of the germ tubes of Gibberella zeae*

Temperature, degrees C.	Time in hours			
	6	24	48	72
	Average length of germ tubes in microns[a]			
4	0.0	g	38.9	137.8
8	0.0	g	146.3	.....
12	0.0	51.6	.....	.....
16	10.6	131.8	.....	.....
20	31.6	.....	.....	.....
24	65.2	.....	.....	.....
28	111.1	.....	.....	.....
32	63.2	.....	.....	.....

[a] g = short knobby germ tubes just beginning to protrude from the conidia.

information on the effect of temperature on germination as well as on growth. Measurements on germ tube length made at the end of 6 hours showed that the most rapid elongation of the germ tubes occurred at 28° C. The germ tube measurements from conidia germinated at the different temperatures coincide closely with the data on mycelial growth.

EFFECT OF INOCULUM DOSAGE ON DISEASE SEVERITY

The studies on the effect of inoculum dosage on disease severity were made to determine the approximate number of spores to apply to the plants in the remainder of the experiments on disease development. Initial experiments indicated considerable variation in the amount of infection established and in the number of heads and spikelets killed when various quantities of conidia were applied to plants. To standardize the procedures, several experiments were conducted on the effects of inoculum dosage on disease development. The results of two of these experiments are presented in figure 1, B. The amount of disease development was not the same in the two experiments, probably because the plants treated in the first experiment were exposed for 48 hours at 25° C. in the humidity tent, whereas the other series received an additional exposure of 12 hours at the same temperature. Since the application of two million conidia per pot resulted in a high percentage of infection in 48 hours at 25° C. and in 100 per cent of the heads and spikelets becoming infected and killed with 60 hours' exposure, this conidial dosage was selected as a standard for use in the remaining experiments.

RELATION OF HEAD MATURITY TO DISEASE DEVELOPMENT

The studies on the relation of inoculum dosage to disease development indicate that there is a difference in susceptibility of wheat heads to *Gibberella zeae* infection during the various stages of head maturity. Several experiments were conducted to determine the relative susceptibility of the heads and spikelets to infection from the boot to the past-flowering stage of development.

In order to determine the susceptibility in the various stages of development, several wheat heads were tagged and records were obtained on the exact stage of development of each of the spikelets on each head prior to inoculation with *Gibberella zeae*. After inoculation the plants were subjected to continued wetness for 48 hours at 25° C. Records on infection were taken 3 days after inoculation and on killed spikelets 5 days later (Table 3). In another experiment, heads in the before-flowering and flowering stages of development were tagged and observed before and after inoculation. The results were the same as those obtained in the previous experiment. No infection was observed on spikelets which had not blossomed prior to the time when the plants were removed from the humidity tent, except in the case of two spikelets which were infected at the base. The infection of one of these appeared to have resulted from contact with

an infected extruded anther from the spikelet just below it. The anther in this case was lodged between the spikelet and the rachis. On the heads showing partial anthesis, only those spikelets which had blossomed were infected. In this respect, the results appear to support the theories of Pugh, Johann, and Dickson (13) who concluded that the progress of infection of the spikelets was dependent upon anthesis. Apparently wheat heads are very resistant to infection prior to flowering.

Atanasoff (2) and Pugh, Johann, and Dickson (13) found that plants inoculated in the flowering stage were more susceptible to infection than those inoculated in the later stages of development. In order to obtain further information regarding the comparative susceptibility of plants in the flowering, after-flowering, and past-flowering stages of development, 18 pots of wheat were inoculated in each of these stages. Six pots from each set of 18 were placed in each of the 20°, 25°, and 30° C. humidity tents. The results from the 36-hour series are illustrated in figure 2, A. In gen-

TABLE 3.—*Comparative susceptibility of plants in different stages of heading to Gibberella headblight*

Stage of development at inoculation	Heads		Spikelets		
	Inoculated	Infected	Inoculated	Infected	Killed
	Number	*Per cent*	*Number*	*Per cent*	*Per cent*
Boot	23	0	131	0	0
Before flowering	36	53	378	6	4
Flowering	20	100	188	79	84
After flowering	6	100	46	98	87
Past flowering	66	100	549	100	100

eral, the plants inoculated in the past-flowering stage were more susceptible to infection and headblight development than those inoculated in the flowering stage. For example, the series exposed to 36 hours of continued wetness at 25° C. had 54, 68, and 98 per cent of the spikelets infected and 14, 74, and 94 per cent of the spikelets killed in the flowering, after-flowering, and past-flowering stages, respectively.

A difference was noted in the size of the kernels produced on plants inoculated in the various stages and subjected to optimum conditions for infection and blight development. Few or no kernels were produced on those plants inoculated in the flowering stage. The kernels from plants inoculated in the after-flowering and past-flowering stages were progressively larger. The kernels from plants inoculated during and after the flowering stage and up to the dough stage were always small and shrivelled.

The degree of symptom expression varied with the stage of development at the time infection occurred. Heads of wheat infected in the flowering or after-flowering stages were marked by deep brown to slightly reddish or creosote-colored lesions. These symptoms first appeared on the lemma, then on the base of the spikelets, the rachis, and the culm. Heavily infected spikelets, killed as a result of infection, gradually lost the deep

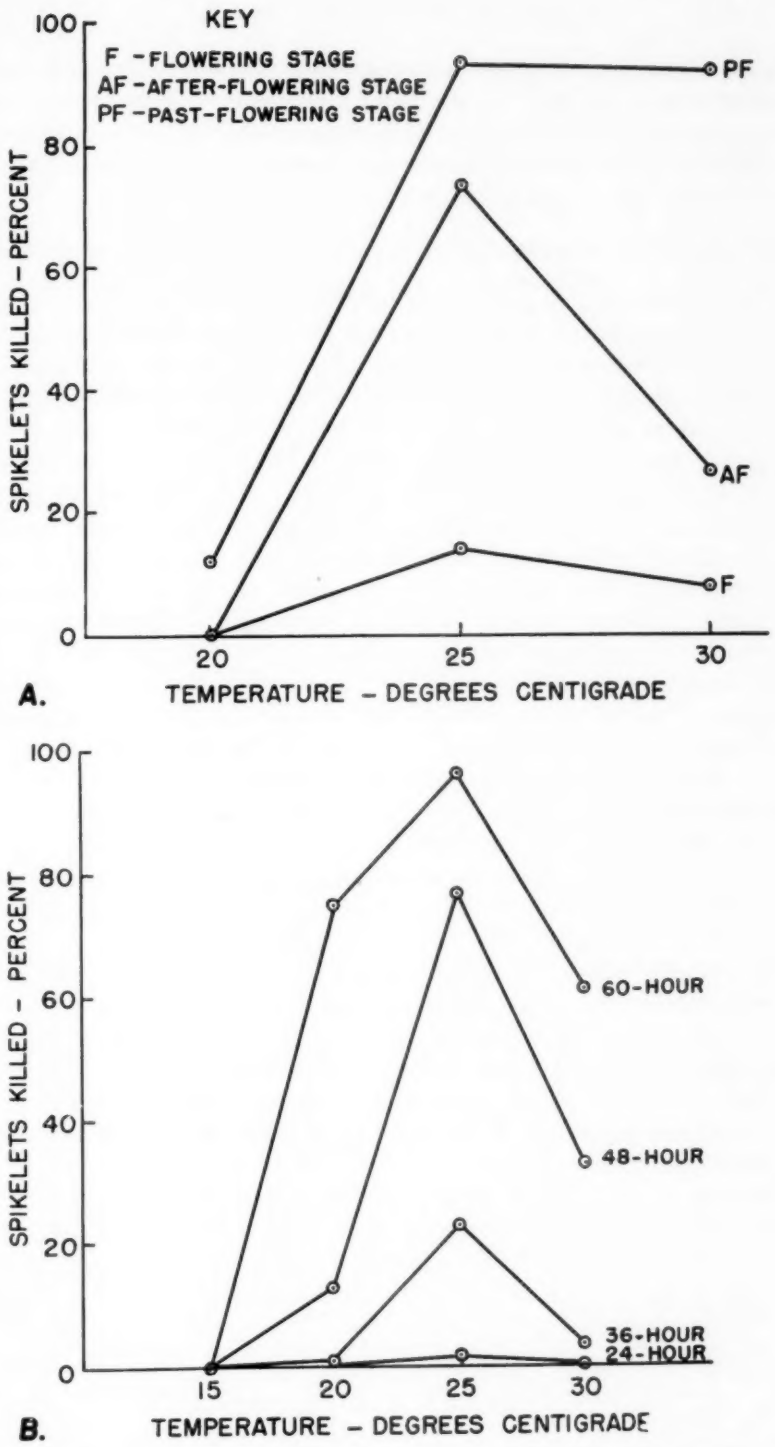

FIG. 2. A. The comparative susceptibility of Newthatch wheat in different stages of development to infection by *Gibberella zeae*. Plants were exposed 36 hours to continued wetness after inoculation. B. The relation of temperature and period of continued wetness to Gibberella headblight. Plants inoculated in the past-flowering stage.

brown discoloration and became straw-colored. Generally, the dark brown discoloration was retained on the culm and rachis. Those plants inoculated in the past-flowering stage of development showed no distinct coloration. Instead, the infected heads and spikelets became bleached and straw-colored.

INFLUENCE OF AIR TEMPERATURE AND MOISTURE ON DISEASE DEVELOPMENT

Infection Studies

Several experiments were conducted in which air temperature and moisture were studied in relation to the infection of wheat heads inoculated with *Gibberella zeae*. Only three of these will be mentioned specifically since the results obtained coincide very closely with those in the other experiments. The experiment on the effect of these factors on the development of headblight of wheat in the flowering, after-flowering, and past-flowering stages of development was mentioned in the preceding section (Fig. 2, A). In the second experiment, 72 pots of wheat in the past-flowering stage of development were inoculated in lots of 8 at regular intervals over a 60-hour period. Two pots from each lot were immediately placed in each of the four humidity tents maintained at 15°, 20°, 25°, and 30° C., respectively. All the pots were removed to the greenhouse bench at the end of the 60-hour period. Thus it was possible to make comparisons of inoculated plants exposed for 6, 12, 18, 24, 30, 36, 42, 48, and 60 hours to continued wetness at the 4 temperatures. The results are illustrated in figure 2, B. In the third experiment, 96 pots of wheat in the flowering stage were all inoculated at one time and distributed equally among the 4 humidity tents maintained at the same temperatures as in the experiment just mentioned. Three pots were removed from each tent after 6, 12, 18, 24, 36, 48, 60, and 72 hours (Table 4).

The period of continued wetness appeared to be an important factor in the determination of the optimum temperature for infection and headblight development. Long exposures at 25° and 30° C. resulted in complete killing of all inoculated wheat heads. Plants given a shorter exposure to continued wetness at 25° C. always developed the most severe headblight. The disease was less severe at 20° than at 25° and 30° C. and only a trace of infection occurred at 15° C.

There appeared to be a definite period during the exposure of inoculated plants to continued wetness at temperatures from 20° to 30° C., in which there was a considerable increase in infection and disease severity. Infection, in this case, is based upon the visible number of infected spikelets, whereas disease severity is based upon the number of spikelets killed. For example, those plants inoculated in the flowering stage and incubated at 25° C. showed an increase in infection from 18 per cent with 36 hours to 77 per cent with 48 hours of exposure, and the disease severity increased from 19 to 64 per cent during this same period. At 20° C. the increase in infection was from 30 per cent with 60 hours to 80 per cent with 72

hours of exposure and disease severity increased from approximately 13 per cent to 96 per cent during the same period (Table 4). Similar results were obtained with plants inoculated in the past-flowering stage (Fig. 2, B). This definite period, which may be referred to as the "critical exposure period" for infection and headblight development, was not so noticeable at 30° C.

TABLE 4.—*Gibberella headblight resulting from exposure of plants, inoculated in the flowering stage, to different periods of continued wetness at different temperatures*[a]

Temperature, degrees C.	Exposure period	Heads	Spikelets		
			Total	Infected[b]	Killed[c]
	Hours	*Number*	*Number*	*Per cent*	*Per cent*
15	18	29	253	0.0	0.0
	24	29	226	0.0	0.0
	36	26	217	0.0	0.0
	48	27	248	1.6	2.0
	60	29	244	0.0	0.0
	72	28	241	0.0	1.2
20	18	29	234	1.3	1.3
	24	28	273	2.9	5.9
	36	27	239	4.2	6.3
	48	27	214	5.2	8.0
	60	28	239	30.0	12.6
	72	27	253	80.6	68.8
25	18	29	212	2.4	6.6
	24	27	237	5.5	2.5
	36	26	232	18.5	19.0
	48	29	267	77.1	64.0
	60	27	243	83.0	76.2
	72	23	223	95.6	84.9
30	18	26	189	0.0	0.0
	24	28	232	0.0	0.0
	36	27	221	2.6	3.2
	48	25	232	27.1	27.1
	60	23	196	61.2	29.6
	72	26	213	86.4	.....[d]

[a] There were 3 replications for each treatment. No infection resulted after 6 and 12 hours of exposure.
[b] Recorded 6 days after inoculation.
[c] Recorded 12 days after inoculation.
[d] Some plants were killed by foot-rot.

The importance of the interaction of moisture, temperature, and stage of host development to infection and resulting headblight may readily be seen by comparing the results in figure 2, B, with those in table 4. The results from these experiments represent a comparison of the past-flowering and flowering stages of development. Thus, at 25° C. the 60-hour exposed series had 13 per cent more infection and 20 per cent more spikelets killed in the past-flowering than in the flowering stage. With shorter exposures, the differences were less. This is further illustrated in figure 2, A. From these results it appeared that the degree of headblight de-

velopment is dependent upon the period of exposure to moisture, the air temperature, and the stage of host development as well as the quantity of inoculum.

Period of Incubation

Pugh, Johann, and Dickson (13) noted that the length of the incubation period was influenced by temperature and that higher temperatures (32° C.) accelerated the appearance of symptoms. In the present studies, the incubation period was determined by making daily observations from the time the plants were removed from the tents until symptoms became visible (Table 5). Plants inoculated in the flowering stage and exposed for 36 hours at 25° C., showed symptoms in 2 days, whereas plants from the same series which were exposed for 24 hours did not show any sign of infection until the fifth day. Similarly, the 48-hour exposed series at 30° C. had an incubation period of 2.5 days as compared to 6 days for those receiving only 36 hours' exposure. There was a gradual decrease in the length of

TABLE 5.—*Incubation periods for plants inoculated in the flowering stage with Gibberella zeae and exposed to different periods of continued wetness at 15°, 20°, 25° and 30° C.*[a]

Temperature, degrees C.	Hours of exposure					
	18	24	36	48	60	72
	Incubation period—days					
15	.....	.....	.....	.....	.....	.....
20	7	6	6	5	4	3
25	6	5	2	2	2	2
30	.....	.....	6	2.5	2.5	2.5

[a] Plants were incubated on the greenhouse bench at 25° C. after removal from the humidity tent.

the incubation period for those plants inoculated in the flowering stage and exposed at 20° C. It appears that the length of the incubation period was determined entirely by the establishment of the fungus in the host tissues at the time the plants were removed from the tents and placed on the greenhouse bench, and that temperature and moisture were the controlling factors in the initial establishment of this infection.

Symptom Expression

Aerial mycelium was consistently observed on the surface of the glumes at the time the plants were removed from the humidity tents. In order to determine if there was any relationship between its presence and the length of exposure to continued wetness at various temperatures, records were taken on the presence or absence of mycelium on inoculated wheat heads at the time they were removed from the tents. In general, the amount of aerial mycelium increased with the length of exposure at any one temperature. There was considerably more at 30° than at 25° C., but less at 20°

C. The presence of aerial mycelium on a spikelet was not a sign of infection of that particular spikelet. It was consistently noted that mycelium growth occurred most frequently in degenerating anther tissue. These observations show that temperature and moisture, as well as stage of development, influence the growth of aerial mycelium on the surface of the spikelets.

Differences were also noted in the degree of color expression. Those plants exposed at 30° C. produced the least discoloration. The deepest browning occurred on those plants exposed to temperatures of 20° and 25° C. For those in the latter group, the symptoms appeared to be more pronounced in those plants exposed the longest to continued wetness.

Discontinuous Wetness

To simulate the variable environment occurring under natural field conditions, inoculated plants were exposed to discontinuous or intermittent

TABLE 6.—*Effect on resulting headblight on plants inoculated in the flowering stage with Gibberella zeae, of no exposure and a 6-hour initial exposure to continued wetness at 25° C. followed by 4 and 8 days of drying and a second wetness period of 48 hours at 25° C.*

Exposure period	Drying period	Heads	Spikelets		
			Total	Infected	Killed[a]
Hours	*Days*	*Number*	*Number*	*Per cent*	*Per cent*
0	4	13	111	15.3	10.0
	8	15	150	36.7	15.3
6	4	17	191	21.5	9.4
	8	19	191	33.0	19.9
48	0	15	144	93.0	52.1

[a] Recorded 10 days after inoculation.

wetness in the greenhouse. In one experiment, 10 pots of wheat were inoculated in the flowering stage and treated as follows: (a) Two pots were exposed to continued wetness for 48 hours at 25° C. immediately after inoculation; (b) four pots were exposed 6 hours to continued wetness at 25° C. and (c) four were immediately placed on the greenhouse bench in a relatively dry environment. Four and 8 days later, 2 pots from series b and 2 pots from series c were subjected to 48 hours of continued wetness at 25° C. (Table 6). It was found that drying periods of 4 or 8 days immediately after inoculation or a short wet period of 6 hours followed by a 4- or 8-day dry period resulted in a considerable reduction in infection and disease severity. Identical conclusions were made from the results obtained in two similar experiments.

Sporulation

The preceding sections dealt principally with the initial establishment of infection and subsequent disease development as influenced by temperature and moisture. This section is devoted to a study of the relation

of these factors to sporulation on wheat heads, a feature which is important to the secondary spread of the fungus under field conditions. In the first set of experiments, the plants were inoculated and exposed for 48 hours to continued wetness at 25° C. and then incubated on the greenhouse bench for a week until the symptoms were well developed and some of the spikelets killed. At the end of this period several of the plants were re-exposed to continued wetness for indefinite periods. Wheat heads were removed at regular intervals, washed in water, and conidial counts made to determine the number of conidia present on the heads. The results from one of these experiments are presented in table 7. The second set consisted of plants which were inoculated and placed immediately in the tents for indefinite periods. Heads were removed from these plants at periodic intervals and the number of conidia determined. The results from a representative experiment are presented in table 8. In this way a good estimate of the number of days required for conidia to be produced and the rate at which they were produced was obtained.

TABLE 7.—*Sporulation of Gibberella zeae on infected heads of plants exposed for different lengths of time to continued wetness at 15°, 20°, 25° and 30° C. in the humidity tents*[a]

Temperature, degrees C.	Hours of exposure to moisture[b]		
	24	48	72
15	0.00	0.34	2.62
20	0.28	3.10	11.88
25	0.63	4.27	14.11
30	0.31	1.63	5.11

[a] Values represent average number of conidia, in millions, from 3 heads.
[b] No conidia were present on those heads removed after 12 hours' exposure.

Differences in sporulation were encountered on the heads of wheat in which infection was already well established when they were placed in the humidity tents and exposed to continued wetness for various periods at temperatures of 15°, 20°, 25°, and 30° C. (Table 7). A few conidia were produced after 24 hours of exposure at the three higher temperatures. Conidia were produced in 48 hours on the heads exposed at 15° C. In a previous experiment in which heads were removed from plants after exposures of 12, 18, and 24 hours, conidia were produced only on those removed after 24 hours. The best temperatures for sporulation were 20° and 25° C. At these temperatures an average of 3–4 million conidia was produced on each head in 48 hours and 12–14 million in 72 hours.

Slightly different results were obtained in those studies on sporulation involving plants exposed continuously to moisture from the time of inoculation (Table 8). In this series only the 20°, 25°, and 30° C. temperature tents were used. Initial experiments indicate that at least 3 days would be necessary for conidia to be produced on wheat heads treated in this manner and exposed at any of the above mentioned temperatures. In this

experiment (Table 8) a few conidia were observed at 20° C. 5 days after inoculation, and an average of 5 million conidia per head after 6 days. At 25° C., an average of 36 million conidia per head was produced 6 days after inoculation. Like results were obtained with Cadet wheat plants that received similar treatments. It is evident that a longer time was required for conidia to be produced on plants inoculated and exposed continuously to moisture than on plants which already had disease lesions present on their heads at the time they were subjected to continued wetness. This is probably because the fungus must establish itself in the host tissue before it can begin to reproduce.

To obtain further information on sporulation of *Gibberella zeae* on infected wheat heads under different temperature conditions, such heads were removed from the plants and placed in water. The heads were divided into seven lots and each lot put in a small humidity chamber prepared by

TABLE 8—*Sporulation of Gibberella zeae on heads of wheat inoculated in the flowering stage and exposed to continued wetness at 20°, 25°, and 30° C. in the humidity tents*[a]

Temperature, degrees C.	Hours of exposure to moisture			
	72[b]	96	120	144
20	0.00	0.00	0.15	5.00
25	0.58	1.60	8.19	36.82
30	0.27	1.51	13.44	17.84

[a] Values represent average numbers of conidia, in millions, from 3 wheat heads.
[b] No conidia were present on those heads removed after 48 hours of exposure.

lining a 4000-ml. beaker with moist paper toweling and covering it with a glass plate. The heads were incubated at 4° intervals from 8° to 32° C. Three heads were removed from each set at 12-hour intervals for the first 60 hours and at two subsequent 24-hour intervals. The total number of conidia present on each sample was determined. A few conidia were produced within 48 hours at 24° and 28° C. After 3 or 4 days, numerous conidia were being produced at temperatures from 20° to 32° C., and a few at 16° C. It is evident from these studies that high temperatures favor sporulation as well as infection and disease development.

DISCUSSION

It was apparent that the isolate used in these studies was similar in its temperature requirements to those isolates used by Dickson (4), MacInnes and Fogelman (9), Tu (17), and Tanja (16). Collectively, these authors found that the cardinal temperatures for the growth of *Gibberella zeae* were approximately 3°, 24–28°, and 32–36° C. In the present studies the cardinal temperatures for growth were approximately 4°, 28°, and 32° C., with no growth at 36° C. Further evidence of this agreement was obtained by comparing the results on infection studies obtained by Pugh,

Johann, and Dickson (13) with similar observations made in the present studies.

Many factors enter into a study on the relationship of the environment to disease development. In the present studies on the development of wheat headblight caused by *Gibberella zeae*, some of the more important factors encountered which influenced the amount and severity of disease development were the inoculum dosage, the stage of host maturity, the period of exposure of the plants to a moist atmosphere, and the air temperature at the time of exposure. The factors relating to the variation of the pathogen were not considered in this study, although they, too, are of utmost importance in a study of this type (17). However, all of these factors are sufficiently important to be considered not only in greenhouse experimentation but in field experimentation as well, whether it be by the plant pathologist or the plant breeder.

Inoculum dosage was shown to affect the amount and severity of wheat headblight. In the field this would entirely depend upon the prevalence of the pathogen on crop residues and as a result, it would be affected by the control measures applied. MacInnes and Fogelman (9), Dickson and Mains (5), Muncie (11) and others suggest that Gibberella headblight (scab) can be kept to a minimum by using clean seed, practicing field sanitation, and by using proper crop rotation.

The stage of host maturity at the time of incubation and infection was another factor of considerable importance. This had already been mentioned by Pugh, Johann, and Dickson (13) who noted that wheat was most susceptible to infection in the flowering stage. The results from this study, however, indicated that there was an increase in susceptibility of wheat heads to infection as the plants increased in maturity from the flowering to the past-flowering stage. This discrepancy with previous work may have resulted from differences in the method of incubation or in the method of evaluating infection. Pugh, Johann, and Dickson (13) incubated their plants continuously in chambers maintained at approximately 70 per cent relative humidity and based their conclusions on the greater reduction in kernel weight and on the larger number of infected seeds which they obtained from plants inoculated in the flowering stage. The results presented in this paper were based on the number of infected and killed spikelets. If the seeds from the 36-hour series at 25° C. had been weighed, no doubt those from the flowering stages would have weighed more than those from the past-flowering stage. However, had the 60-hour series at 25° C. been used as a basis for comparison, then higher yields would doubtless have been obtained from those plants inoculated in the past-flowering stage since there were few or no kernels produced on those plants inoculated in the flowering stage. This would indicate that the relative susceptibility of the plants in the different stages of development was dependent upon the length of exposure to continued wetness. However, in all the experiments on Gibberella headblight reported upon in this paper, it was consistently

noted that the plants inoculated in the past-flowering stage were more susceptible than those given a similar treatment in the flowering stage.

Several workers (2, 3, 5, 8, 9, 10, 12, 14, 15) have mentioned that Gibberella headblight of wheat is favored by periods of wet, humid weather and high temperature during the heading period. They did not, however, determine how long an exposure was necessary for infection. Atanasoff (2) and Christensen, Stakman, and Immer (3) further stress the importance of these conditions to sporulation and secondary spread of the organism. The results presented in this paper confirm the observations made by the above authors as to the important part played by moisture and temperature in the development of Gibberella headblight epiphytotics. Not only do these two factors influence the establishment of infection, but they influence the numbers and the rapidity at which conidia are produced on the infected heads. It would be difficult to estimate the actual numbers of conidia that could be produced within 2–3 days in a field of infected wheat under optimum conditions for sporulation and blight development. The fact that the fungus is capable of establishing itself in the host tissue within 24–36 hours at 25° C., and, in addition, is able to produce one-half million new conidia on each infected wheat head within 72 hours, may be a partial explanation of why the organism is capable of spreading rapidly under favorable weather conditions.

Of special significance to those investigators involved in the control of Gibberella headblight by breeding for disease resistance, was the varying amount of infection and severity of headblight development resulting from the exposure of the inoculated plants to different temperature and moisture conditions during different stages of development. Wheat varieties are ordinarily tested for resistance in the field where conditions vary from day to day and from season to season. Very seldom, under such conditions, are all plants in the same stage of development at the time of inoculation and when favorable conditions for infection and headblight development prevail. Therefore, it would be expected that various degrees of infection may be present and that many plants may escape infection entirely. The conclusion of Christensen, Stakman, and Immer (3) that "Considerable caution is necessary in drawing conclusions from the results of varietal tests unless they are conducted for several years under carefully replicated and controlled conditions," is borne out by this work.

SUMMARY

1. The growth of *Gibberella zeae* mycelium and the germination of the conidia took place at temperatures from 4° to 32° C., with the most rapid growth of the mycelium and the elongation of the germ tubes occurring at 28° C. The most rapid germination occurred at 28° and 32° C.

2. The most rapid production of conidia occurred at 28°–32° C. on agar media; but on longer incubation, more conidia were produced at 32° C. than at any other temperature. Few conidia were produced at and

below 16° C. and none were produced at 36° C. Mainly 5-septate conidia were formed at 20°–24° C., whereas 2-septate conidia predominated at 32° C.

3. Conidia were produced on infected heads of plants exposed to continued wetness at temperatures from 15° to 30° C. The maximum number was produced on those heads exposed at 25° C. with high production occurring at 20° C. At 25° C., conidia were produced within 24 hours on plants which already had infection well established at the time the plants were exposed to continued wetness, whereas 72 hours of exposure were required for the formation of conidia on plants exposed to continued wetness from the time they were inoculated. An average of 14 million conidia per head was produced in 3 days after re-exposure of the infected plants and 36 million were produced in 6 days on plants exposed continuously after inoculation.

4. The amount of infection and disease severity increased with an increase in the amount of inoculum up to 1.0–2.0 million conidia per pot of 10 wheat plants.

5. Little infection occurred on wheat heads inoculated prior to flowering. The order of increased susceptibility of wheat to Gibberella headblight was from the flowering to the past-flowering stages, with decreasing susceptibility at late stages of seed development.

6. The best temperature for infection and headblight development was 25° C. Little or no infection occurred at 15° C. More rapid infection occurred at 30° than at 20° C., but after 60–72 hours' exposure to continued wetness the amount of infection and disease development was approximately the same at these two latter temperatures.

7. The period of exposure to continued wetness favoring maximum infection and disease severity varied with the stage of host development and the temperature during exposure. The period of exposure necessary for infection was the shortest at 25° C., becoming progressively longer at the lower and higher temperatures. The exposure period necessary for infection was the shortest (at any one temperature) for those plants inoculated in the past-flowering stage and longest for those inoculated in the flowering stage.

8. The critical exposure periods for disease severity were, for plants inoculated in the past-flowering stage, from 48 to 60 hours at 20° C. and 36 to 48 hours at 25° C.; for plants inoculated in the flowering stage, 60 to 72 hours at 20° C., and 36 to 48 hours at 25° C.

9. Symptoms became visible 2 days after inoculation on plants inoculated in the flowering stage and exposed to continued wetness for 36 hours at 25° C. At 20° and 30° C. with the same exposure period, no symptoms were visible until after 6 days' incubation. In general, longer incubation periods were encountered at the lower and higher temperatures and with shorter periods of exposure to continued wetness at any one of these temperatures.

10. A dry period of 4–8 days immediately following inoculation of plants or following an initial 6-hour exposure of inoculated plants to continued wetness, resulted in a reduction in disease severity in comparison with that obtained on plants receiving no intermediate drying period after inoculation.

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RELATION OF SOIL FUMIGATION, NEMATODES, AND INOCULATION TECHNIQUE TO BIG VEIN DISEASE OF LETTUCE

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There are only a few known plant viruses whose occurrence in nature seems to be intimately associated with the soil. These viruses, or the diseases they cause, are frequently referred to as being soil-borne. The best known virus of this type is probably that causing mosaic of wheat (10). Big vein of lettuce and some oat mosaics (13) are also soil-borne. These diseases seem to have the following characteristics in common: They occur in nature only when susceptible plants are grown in soil containing the causative agent; vectors, if they exist are not known; infested soils can be made non-infective by treatment with formaldehyde or by partial steam sterilization; they are more common in heavy soils than in light soils; the causative agent persists in the soil for long periods of time in the absence of known host plants; infection under natural conditions appears to take place through the roots of susceptible plants.

The suggestion that nemtaodes might be possible vectors of the soil-borne virus causing winter wheat mosaic was made by McKinney (11) and Johnson (7). Johnson (7) found that the symptoms of wheat mosaic did not develop in wheat grown in soil treated with certain fumigants. He suggested that the action of the fumigants was probably on a vector rather than on the virus. McKinney (12) also suggested that wheat-mosaic-infested soil contained some important contributing factor in addition to virus and that it was possible that the virus occurred in some soil-inhabiting organism that served as a vector.

The present investigation was undertaken to ascertain the relationship of nematodes to the transmission of lettuce big vein. Experiments with artificial transmission were undertaken to determine the probable action of fumigants on big-vein-infested soil and to clarify results obtained in nematode transmission tests. Observations were also made on some physical properties of the virus.

REVIEW OF LITERATURE

Lettuce big vein disease was first described by Jagger and Chandler (5) from lettuce grown in the Imperial Valley of California. Jagger and Chandler (5) stated that the causal agent of big vein was apparently soil borne since symptoms of the disease did not develop in plants grown in soil treated with formaldehyde or by partial steam sterilization. It was found that the incidence of the disease was not influenced by the addition of certain chemicals and fertilizers to infested soil. They also reported that the causative agent could persist in soil for long periods of time in the field and in the greenhouse.

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The effect of soil moisture on the development of the symptoms of lettuce big vein disease was investigated by Pryor (14). He found that high soil moisture favored the development of the symptoms in lettuce, and that vigorously growing plants seemed to be most readily affected by the disease. He reported that the earlier a plant developed the disease, the smaller its final size. Thompson and Doolittle (17) observed that lettuce appeared to develop symptoms of big vein disease more readily in air temperatures of 7.2° to 15.5° C. than in air temperatures of 10° to 23.8° C. Pryor (15) found that the optimum soil temperature for the development of big vein symptoms was approximately 22° C. However, he reported that lettuce plants developed symptoms at all soil temperatures ranging from 18° to 30° C.

Rawlins and Tompkins (16) obtained negative results in the artificial transmission of big vein by leaf-rubbing with juice from the foliage of infected plants, with and without carborundum as an abrasive. Pryor (15), reporting the results of investigations conducted in 1941, also obtained negative results in experiments involving artificial transmission of the disease. He concluded from the results of numerous trials that big vein is rarely, if ever, transmitted by the seed of infected plants. Thompson, Doolittle, and Smith (18) were unable to transmit big vein by any of the methods commonly used to transmit plant viruses, but Doolittle and Thompson (2) reported a year later that they had been successful in artificially transmitting lettuce big vein virus. They succeeded in obtaining infection in 56 out of 76 plants inoculated by rubbing leaves or pricking freshly extracted root juice from big-vein-infected plants into the leaves or stems of healthy plants. Parallel inoculations with the juice from the mottled leaves of the plants whose roots were used as inoculum produced no infection. Eighty control plants remained healthy throughout the experiment.

Several insect species have been tested as possible vectors of lettuce big vein disease. Thompson, Doolittle, and Smith (18) obtained no evidence of big vein transmission with *Aphis gossypii* Glover, *Macrosiphum solanifolii* (Ashmead), and *Trialeurodes vaporariorum* (Westwood). A low percentage of transmission was reported using as vectors *Myzus persicae* (Sulzer), *Myzus circumflexus* (Buckton), and an undescribed *Macrosiphum* species. They were apparently able to secure some transmission of lettuce big vein virus with the root aphid *Pemphigus lactucae* (Fitch). It is pointed out by these authors that the presence of big vein disease in the control plants of their experiment might be explained on the basis of the migratory habits of this aphid. Pryor (15) did not obtain consistent transmission of lettuce big vein virus by the aphids, *Macrosiphum solanifolii* (Ashmead), *Myzus solani* Kaltenbach, and *Myzus persicae* (Sulzer). The root aphid, *Pemphigus lactucae* (Fitch), was not present in his greenhouse and he suggested that other vectors might exist.

Soil transmission of lettuce big vein virus has been reported by Jagger and Chandler (5), Thompson, Doolittle, and Smith (18), and Pryor (15).

Pryor (15) prepared leachates from big-vein-infested soil and found that filtering this leachate through a medium Mandler filter removed the causative agent. Unfiltered leachate added to big-vein-free soil produced big vein symptoms in four of 84 plants. That leaching the soil with large amounts of water does not reduce the infectivity of the soil was reported by Jagger and Chandler (5) and Pryor (15). Pryor (15) diluted big-vein-infested soil one part to 800 parts of autoclaved soil and found the incidence of disease was only slightly reduced by the dilution. He reported that air-dry soil remained highly infective for at least eight years.

MATERIALS AND METHODS

The investigations on the transmission of lettuce big vein virus were conducted in the greenhouse and in the laboratory. Big-vein-infested soil was secured from infested lettuce fields in the Salinas Valley. In certain experiments infested soil was mixed with greenhouse soil at a ratio of one to one. This was considered satisfactory procedure since Pryor (15) has reported that such dilutions do not decrease the infectivity of the soil.

Handling of Nematodes. The nematodes tested as possible vectors of big vein virus were collected from soil in which diseased lettuce plants were growing. They were removed from the infested soil by the procedure outlined by Cobb (1). This consisted of mixing soil containing the nematodes with water. The soil-water mixture was passed through a series of graded screens and finally through a piece of finely woven bolting silk. The silk used in these experiments had apertures of 30 to 50 microns in diameter and retained large numbers of the small nematodes present in the soil. The residue remaining in each screen was placed in tap water and examined under a dissecting microscope. Nematodes were removed from the residue by means of a fine bamboo splinter. Each nematode was handled singly to avoid transfer of the debris present in the screening residue. Nematodes were transferred to a small watch glass containing distilled water. After the nematodes to be used in any particular test had been collected, they were transferred to heat-treated, autoclaved or fumigated soil by means of a small pipette. The treated soil was contained in four-inch or six-inch greenhouse pots. After inoculation of the soil with nematodes, the pots were placed on wooden benches in the greenhouse and lettuce seed was planted directly into the soil or a lettuce seedling grown in virus-free soil was transplanted into each pot. Lettuce in these tests was grown in the inoculated soil at least 60 days before the final results were recorded. In several experiments plants were held under observation more than 90 days before they were discarded. In most instances plants were examined daily beginning 20 days after seeding or transplanting of the lettuce for evidence of the development of big vein symptoms.

Securing Virus-free Soil. The soil used in nematode and artificial transmission investigations was treated by one of three methods to make it free from big vein infestation. The first experiments involved the use of

soil that had been heat-treated in a box type electrically heated soil sterilizer. In subsequent experiments the soil was autoclaved at 17 lb. steam pressure for at least four hours or fumigated with one ml. of chlorpicrin per gal. (231 cu. in.) of soil. The latter two methods proved to be more effective in eliminating big vein from the soil.

Methods of Artificial Inoculation. Artificial transmission experiments were undertaken using the carborundum leaf-rubbing method described by Rawlins and Tompkins (16). In some tests root juice containing the virus was rubbed on the leaves of healthy plants with thumb and forefinger. In the more exacting tests the infective juice was rubbed on the leaves, in the presence of carborundum, with a small cotton swab soaked in the infective juice.

Soil Fumigation. Fumigation tests to determine the effect of soil fumigants on big-vein-infested soil were carried out using infested soil from the field. Prior to fumigation the soil was thoroughly mixed and placed in one-gallon jars. The desired amount of fumigant was introduced into the jar with a pipette and the lid of the jar immediately put in place. The soil was retained in the closed jar for a 48-hour period and then transferred to six-inch greenhouse pots. An aeration period of six days was allowed before lettuce seedlings grown in disease-free soil were transplanted into the fumigated soil. In some experiments it was necessary to make slight changes in the procedures outlined above. These are discussed in detail in connection with the particular experiments involved.

TRANSMISSION TESTS WITH NEMATODES

Some nematodes have feeding habits that might be favorable for the transmission of a virus. Nematodes belonging to the families Tylenchidae and Aphelenchidae possess a stylet that is frequently used to pierce plant cells during the feeding process. Most nematode species in the two families have a bulb-like muscular metacorpus located in the median portion of the esophagus. This bulb is equipped with a triradiate valve which serves as a sort of pump to suck out the contents of cells punctured by the stylet. Linford (8) has observed that several nematode species regularly eject secretions of the esophageal glands during the feeding process. This appears to be essentially the same process by which insect vectors are thought to transmit plant viruses. An investigation of the relationship of nematodes to the transmission of lettuce big vein virus was undertaken because of the apparent underground mode of infection, because no previous investigation had been made regarding the possibility that some nematode might be a vector of big vein virus.

Prior to inoculating virus-free soil with nematodes, samples of big-vein-infested soil were examined to determine the nematode species commonly associated with the roots of diseased lettuce plants. The roots of infected lettuce plants were carefully washed free of soil and examined for the presence of nematodes that might be feeding on the roots. In addition some

roots were stained with acid-fuchsin in lacto-phenol following the procedure suggested by Goodey (3). These roots were carefully examined for the presence of nematodes within the root tissues.

The nematode *Paratylenchus macrophallus* (de Man) was the only species observed feeding on the roots of lettuce. For this reason it was given first consideration as a possible vector of lettuce big vein virus. *Aphelenchus avenae* Bastian, a species frequently observed in the roots of plants, was found to be rather abundant in the soil around the roots on infected lettuce. This species was not observed feeding upon the roots or within root tissue. But because of its abundance in the soil *A. avenae* along with *P. macrophallus* was used in the initial transmission studies. *Acrobeloides bütschlii* (de Man), *Criconemoides mutabile* Taylor, and *Rhabditis monohystera* Bütschli were used separately in subsequent tests. Other nematode species found in

Table 1.—*Number of lettuce plants developing big vein symptoms when grown in heat-treated and autoclaved soil inoculated with nematodes taken from infective soil, in heat-treated soil, autoclaved soil, and in soil infested with big vein*

Treatment	Number of nematodes per plant	Number of plants inoculated	Number of plants infected
Paratylenchus macrophallus	50	10	5[a]
do.	100	29	0
Aphelenchus avenae	20	10	0
Acrobeloides bütschlii	50	20	0
do.	100	20	0
Rhabditis monohystera	100	10	0
Criconemoides mutabile	16	10	0
Miscellaneous nemas	50	20	1[a]
do.	100	20	3[a]
Oligocheat worms	5	20	0
Heat-treated soil	.....	10	2[a]
Autoclaved soil	.....	40	0
Infested soil	.....	34	33

[a] Source of infection uncertain, possibly inadequate heat treatment.

infested soil were not isolated as a single species but were placed in the disease-free soil as miscellaneous lots of nematodes. The following species were present in these lots: *Aphelenchoides parietinus* (Bastian), *Acrobeloides bütschlii* (de Man), *Chiloplacus* sp., *Dorylaimus obscurus* Thorne, *Dorylaimus simplex* Thorne, *Dorylaimus monohystera* de Man, *Panagrolaimus subelongatus* (Cobb), *Rhabditis monohystera* Bütschli, and *Tylenchus filiformis* Bütschli. A few larvae of the sugar-beet nematode, *Heterodera schachtii* Schmidt, were also present in these lots. The inoculation experiments with nematodes were repeated several times and the results are summarized in table 1.

The evidence of nematode transmission of the virus causing big vein of lettuce is mostly negative in these experiments. In no instance was it possible to consistently demonstrate nematode transmission. The relatively few cases of apparent nematode transmission were probably the result of

inadequate heat-treatment of the soil prior to inoculation or to outside sources of infection.

FAILURE TO OBTAIN TRANSMISSION OF LETTUCE BIG VEIN VIRUS BY LEAF INOCULATION

Successful artificial transmission of lettuce big vein virus has been reported only by Doolittle and Thompson (2). Rawlins and Tompkins (16), Thompson, Doolittle, and Smith (18), and Pryor (15) have reported negative results when they inoculated healthy lettuce plants with the juice extracted from the leaves of infected plants. In order to corroborate the results reported by Doolittle and Thompson (2), 20 lettuce seedlings grown in autoclaved soil were inoculated. Freshly extracted root juice of lettuce plants infected with big vein virus was rubbed with the thumb and forefinger, in the presence of carborundum, on the leaves and cotyledons of the seedlings. Twenty lettuce seedlings grown in autoclaved soil were transplanted into big-vein-infested soil and 20 into autoclaved soil. Nine of the 20 inoculated seedlings became infected with big vein virus and 19 of the 20 seedlings transplanted into infested soil developed symptoms. All of the 20 seedlings transplanted into autoclaved soil remained healthy.

The results obtained in the experiment appeared to confirm those reported by Doolittle and Thompson (2), since symptoms of big vein did not develop in the plants growing in autoclaved soil. There was, however, a considerable difference between the incubation period of about 79 days for the inoculated plants in the present experiment and the incubation period of 45 days reported by Doolittle and Thompson (2). It does not appear probable that this difference in incubation time can be attributed to variation in the environmental conditions of the experiments inasmuch as the average incubation period of 40 days for plants grown in big-vein-infested soil is close to the incubation period of 45 days reported by Doolittle and Thompson (2) for lettuce grown in big-vein-infested soil.

The long period of time required for leaf-inoculated lettuce plants to develop symptoms (79 days) gave rise to doubt as to the actual avenue of entrance of the virus into the plants. In this experiment no precautions were taken to prevent contact of the inoculated foliage with the autoclaved soil in which the plants were growing. The leaves of the inoculated plants were not washed after they had been rubbed with the infective root juice and carborundum. However, the plants were irrigated by pouring water on the surface of the soil. This could have served to wash virus present on the external surfaces of inoculated leaves into the soil. In order to obtain more exact information concerning the path by which the virus actually entered the leaf-inoculated plants, additional experiments on artificial transmission were undertaken.

One hundred twenty-five six-inch greenhouse pots were filled with soil and autoclaved for five hours at 17 lb. steam pressure. The pots were then placed in the greenhouse on benches previously drenched with a five per cent

formaldehyde solution. Lettuce seed was planted in the autoclaved soil and after germination the lettuce was thinned so that two seedlings remained in each pot. When these seedlings reached the four-leaf stage, one plant in each of 75 pots was inoculated by rubbing the leaves, in the presence of carborundum, with root juice freshly extracted from big vein lettuce plants. The soil in another lot of twenty pots was inoculated by pouring two ml. of the infective juice onto the surface of the soil. The remaining 25 pots, each containing two plants, were retained as control plants to check the efficacy of the autoclaving treatment. The results of this experiment are given in table 2.

The results clearly demonstrate that the virus causing big vein of lettuce can be transmitted by pouring infective root juice extracts on the soil. Forty-eight out of 50 lettuce plants grown from seed in autoclaved soil that was inoculated with two ml. of infective root juice developed big vein symp-

TABLE 2.—*Number of lettuce plants grown in autoclaved soil developing big vein symptoms as the result of leaf-inoculation and soil inoculation with infective root juice extracted from big vein infected lettuce plants*

Treatment	Number of plants	Number of plants infected	Days for symptoms, average
Autoclaved soil[a]			
Leaf-inoculated	75	17	53.6
Not inoculated	75	11[b]	58.0
Autoclaved soil	50	0	.....
Autoclaved soil 2 ml. juice poured in each pot	50	48	47.3

[a] Two plants per pot, one plant inoculated, the other not inoculated.
[b] Six of these were the only ones of the pair to become infected.

toms. Since lettuce seed was planted directly in the pots containing the autoclaved soil, the virus seems to have gained entrance into the plants in the absence of wounds other than those that might occur naturally during the growth of the lettuce. None of the plants grown from seed planted in untreated autoclaved soil developed big vein symptoms.

In the test in which one plant per pot was inoculated by leaf-rubbing and the other plant was not inoculated, the results obtained can be explained on the basis of infection through the soil. Seventeen of the 75 plants inoculated by leaf-rubbing developed big vein symptoms and 11 of the 75 plants that were not inoculated but grown in the same pots became infected. The average incubation period for the inoculated plants was about 53 days as compared to 58 days for the plants that were not inoculated. In some instances noninoculated plants in pots with inoculated plants developed symptoms before they appeared in the leaf-inoculated ones. Also, in six of the pots only the plant that was not leaf-inoculated developed symptoms. The incubation period was only 47 days where the inoculum was poured into the soil. Presumably this method supplied more inoculum to the roots in a shorter period of time.

The data presented in table 2 indicate that infection with big vein virus through the foliage of lettuce plants is questionable. The most logical explanation for the results seems to be that infection did not develop as the result of leaf-inoculation, but as the result of the virus used in leaf-inoculations gaining access to the soil and infecting the plants by way of the roots. However, since the foliage of the lettuce plants was not protected from the inoculated soil, the possibility of infection through the leaves from contact with the soil was not eliminated. For this reason the experiment was repeated with methods that would prevent foliage contact with the soil.

Forty four-inch greenhouse pots were filled with soil previously fumigated with chlorpicrin at the rate of one ml. per gal. of soil. Lettuce seedlings grown in similar fumigated soil were transplanted into these pots. A cotton pad one-half inch thick was then placed over the surface of the soil contained in each pot so as to prevent contact of the leaves with the soil. This pad was retained in place throughout the experiment. The lettuce

TABLE 3.—*Number of lettuce plants developing big vein symptoms as the result of controlled leaf-inoculation, leaf-inoculation followed by washing of the plants, soil inoculation with five milliliters of infective root juice, and no treatment*

Treatment	Number of plants inoculated	Number of plants infected
Leaf inoculation (soil covered)	40	0
Leaf inoculation (soil not covered, plants washed)	36	31
Soil inoculation with 5 ml. juice (foliage protected)	20	19
Control	20	1

seedlings in this series were inoculated by rubbing the leaves, in the presence of carborundum, with a small cotton swab soaked in infective root juice. These inoculations were carefully made so that an excess of the inoculum was not used. After inoculation of the plants, the pots were placed in saucers and irrigation throughout the experiment was by pouring water into these saucers. Another forty plants were similarly inoculated. One hour after inoculation these plants were washed with a fine spray of water to remove the carborundum residue from the leaves. The plants were washed in a position that allowed the water to drip from the leaves into the soil. The plants in this series were irrigated by pouring water onto the soil in the pots. Twenty lettuce seedlings growing in fumigated soil were inoculated by adding five ml. of infective root juice to the soil surrounding the opening in the bottom of the greenhouse pots. These plants were irrigated from the bottom by water poured into saucers containing the pots. Prior to inoculation the surface of the soil in these pots was covered with a cotton pad to prevent foliage contact with the soil. Twenty seedlings growing in fumigated soil

were retained as controls. The infective root juice used in this experiment was extracted from the roots of 20 lettuce plants showing symptoms of big vein infection. A portion of this juice was used as inoculum in each of the tests in the experiment. The results of this experiment are in table 3.

The results demonstrate that infection with lettuce big vein virus did not take place through the foliage of leaf-rubbed plants and that unless precautions are taken to prevent the inoculum from dripping or washing into the soil, root infection may be thought to be leaf infection. Two methods of soil inoculation are demonstrated, from above and from below, to be effective in experimentally producing the disease.

INFECTION FROM CHOPPED LETTUCE ROOTS ADDED TO THE SOIL

In 1934 Jagger and Chandler (5) reported that lettuce big vein disease was gradually becoming more serious and wide-spread as the result of continuous cropping of the same land with lettuce. Persistence of big vein virus in the soil probably indicates that the virus is returned to the soil as the roots of infected lettuce plants decompose in these fields. It has been demonstrated that the virus of lettuce big vein can enter the plant as the result of root contact with virus in the soil. In order to determine if big vein virus could be introduced into virus-free soil by the roots of infected lettuce plants, a series of pots containing chlorpicrin-fumigated soil were inoculated with thoroughly washed chopped roots of infected plants. The fumigated soil in each of 10 four-inch pots was inoculated with five gm. of infected lettuce roots. Ten pots containing fumigated soil were retained as controls. Ten pots containing untreated soil from the field were inoculated with five gm. of infected roots and 10 pots containing the field soil received no root material. Ten out of 10 lettuce plants grown in fumigated soil inoculated with infected roots developed big vein symptoms. No big vein symptoms were observed in plants grown in the fumigated soil. Eight of 10 plants grown in field soil inoculated with infected lettuce roots developed big vein symptoms. None of the 10 plants grown in noninoculated field soil developed symptoms. These results indicate that virus-free soil can be inoculated with big vein virus by the addition of big vein infected lettuce roots.

SOIL FUMIGATION

Early in the investigation of the possible relationship of nematodes to the transmission of lettuce big vein virus some preliminary experiments were undertaken involving the fumigation of big-vein-infested soil with a nematicide. Approximately one gal. of big-vein-infested soil was exposed for 48 hours in a sealed container to the fumes of dichloropropene-dichloropropane mixture.[2] One ml. of the fumigant was applied per gal. of infested soil. This amount of the fumigant is about the equivalent of 1,000 lb. per acre. After the 48-hour exposure period, the treated soil was transferred to eight

[2] Shell DD, supplied by the Shell Oil Company.

four-inch greenhouse pots and allowed to aerate for six days. Untreated big-vein-infested soil from the same source was placed in six four-inch greenhouse pots. Six days after the fumigation treatment, lettuce seed was planted directly in the soil. In the fumigated soil one out of eight plants developed symptoms of big vein infection. In the untreated big-vein-infested soil, five out of six lettuce plants developed symptoms. The results obtained in this preliminary test indicated that the fumigant was capable of at least partially inactivating the causal agent of big vein of lettuce.

Johnson (7) reported that carbon disulphide, chlorpicrin, methyl bromide, rotenone, and naphthalene prevented the development of the symptoms of winter wheat mosaic in susceptible wheat grown in soil treated with these chemicals. Calcium cyanide and ethylene dichloride gave incomplete control under similar conditions. Johnson (7) suggested that the action of these fumigants was on a vector rather than on the virus. McKinney (10), McKinney, Webb, and Dungan (9), Webb (19), Ikata and Kawai (4), and Johnson (6, 7) have demonstrated that winter wheat mosaic occurs in nature only when susceptible wheat is grown in mosaic-infested soil. Jagger and Chandler (5), Thompson, Doolittle, and Smith (18), and Pryor (15) have obtained evidence that big vein of lettuce also results from root contact with infested soil. It has been shown in the present paper in the section dealing with artificial transmission that a vector is not necessary for the transmission of the virus. It is therefore probable that the action of the dichloropropene-dichloropropane mixture was on the virus *per se*.

To obtain further information on the effect of fumigation on soil infested with big vein virus, additional experiments were undertaken. Dichloropropene-dichloropropane mixture and 20 per cent by weight ethylene dibromide mixture[3] were applied to virus-infested soil at dosage rates of 0.23, 0.46, 0.69, 0.92, and 1.16 ml. per gal. of soil. The soil was treated in one-gallon jars sealed with gas-tight lids. After the proper amount of fumigant was applied to the soil, the jars were closed for 48 hours. The soil was then removed from the jars and placed in six-inch greenhouse pots. Each gallon jar contained enough soil to fill two six-inch pots. Since five jars of soil were treated at each dosage rate, this afforded 10 pots for each dosage applied. Twenty six-inch pots containing untreated virus-infested soil were included in the experiment as controls. Lettuce seedlings grown in autoclaved soil were transplanted into the treated and untreated soil six days after the fumigated soil was removed from the fumigation jars. The results obtained in this experiment are in table 4.

The results indicate that 20 per cent ethylene dibromide mixture had no effect on the development of big vein symptoms in lettuce planted in fumigated soil. The symptoms of the disease developed just as rapidly in soil fumigated with this material as in untreated virus-infested soil; approximately 30 days were required in both instances. Dichloropropene-dichloropropane mixture had a retarding effect on the development of symptoms at

[3] Dowfume W–10, supplied by the Dow Chemical Company.

TABLE 4.—*Number of lettuce plants developing big vein symptoms in virus-infested soil treated with various dosages of dichloropropene-dichloropropane mixture and 20 per cent by weight ethylene dibromide mixture*

Milliliters of fumigant per gallon of soil	Treatments					
	Dichloropropene-Dichloropropane			Ethylene dibromide		
	Number of plants	No. of plants infected	Days for symptoms, average	Number of plants	No. of plants infected	Days for symptoms, average
0.23	10	10	52.8	10	10	31.3
0.46	10	1	73.0	10	10	32.5
0.69	10	0	.....	10	9	29.0
0.92	10	1	74.0	10	10	29.1
1.16	10	0	.....	10	10	30.6
No treatment	20	19	28.4	.....	.....	.....

the lowest dosage applied. At dosages above 0.23 ml. per gal. of soil, only two plants of 40 developed big vein symptoms in soil treated with this material.

In another experiment big-vein-infested soil collected in the Salinas Valley, California, was treated with several fumigants. The method of fumigation was the same as in the previous experiment with fumigants. The following materials were applied to the big-vein-infested soil: Two dichloropropene-dichloropropane mixtures, ethylene dibromide mixture con-

TABLE 5.—*Number of lettuce plants developing big vein symptoms when grown in virus-infested soil treated with various fumigants*

Treatments	Ml. or gm. of fumigant per gallon of soil	Number of plants	Number of plants infected	Days for symptoms, average	Weight of tops in grams, average
Dichloropropene-dichloropropane[a]	0.23	10	8	40.1	22.5
	0.46	10	0	.....	31.7
	0.69	10	0	.....	23.8
Dichloropropene-dichloropropane[b]	0.23	10	8	35.5	21.9
	0.46	10	0	.....	35.0
	0.69	10	0	.....	24.7
Ethylene dibromide[c]	0.23	10	10	29.9	18.5
	0.46	10	10	29.2	21.6
	0.69	10	7	29.1	16.5
Chlorpicrin[d]	0.23	10	0	.....	33.3
	0.46	10	0	.....	47.6
	0.69	10	0	.....	32.5
Benzene hexachloride[e]	0.70	10	10	27.2	17.1
	1.40	10	9	34.5	22.8
	2.80	10	7	30.8	10.0
No treatment	.....	13	13	30.6	.....

[a] Supplied by the Shell Oil Company.
[b] Supplied by the Dow Chemical Company.
[c] 40 per cent by weight supplied by the Dow Chemical Company.
[d] Supplied by the Innis, Speiden, and Company.
[e] Dust containing one per cent gamma isomer supplied by California Spray Chemical Company.

taining 40 per cent of ethylene dibromide by weight, chlorpicrin, and benzene hexachloride dust containing one per cent of the gamma isomer. The dichloropropene-dichloropropane mixtures, chlorpicrin, and ethylene dibromide mixture were applied to the big-vein-infested soil at the rate of 0.23, 0.46, and 0.69 ml. per gal. of soil. The benzene hexachloride dust was applied at the rate of 0.70, 1.40, and 2.80 gm. per gal. of soil. This amount is approximately the equivalent of 5, 10, and 20 lb. of the gamma isomer per acre. The results of this test are in table 5.

The data recorded indicate that chlorpicrin was the most effective of the fumigants tested in inactivating the virus of lettuce big vein in the soil. The results obtained with dichloropropene-dichloropropane mixtures are similar to those obtained in the previous experiment. Ethylene dibromide and benzene hexachloride did not appear to have any effect on the virus in the soil. Lettuce seedlings grew more vigorously and produced larger plants in the soil that was treated with chlorpicrin and the intermediate dosages of dichloropropene-dichloropropane mixtures. Plants grown in virus-infested soil treated with fumigants that prevented the development of big vein symptoms, or delayed the appearance of symptoms, were larger than plants grown in virus-infested soil treated with materials that did not prevent or delay the development of symptoms. The higher dosages, however, appeared to have inhibited the growth of lettuce.

It was assumed in the previous experiments with soil fumigants that the action of the fumigant was on the virus. This assumption is based on the fact that lettuce growing in autoclaved soil becomes infected with big vein when root juice extracts from big-vein-infected plants are added to the soil in the absence of probable vectors. One attempt was made to secure direct evidence for this assumption. Eighty ml. of root juice were extracted from the roots of big-vein-infected lettuce plants. This juice was diluted with an equal quantity of water and centrifuged for one hour at a speed of 3,000 revolutions per minute. After the centrifugation, the supernatant was filtered through a medium Mandler filter. The filtered juice was added to greenhouse soil previously autoclaved for five hours at 17 lb. steam pressure. The filtered, centrifuged juice did not cause the development of big vein symptoms in lettuce seedlings planted in the inoculated soil. Pryor (15) found that water extracts of big-vein-infested soil became non-infective when filtered through a medium Mandler filter.

THERMAL INACTIVATION OF THE VIRUS

This phase of the investigation was originally intended to be a part of the section dealing with nematode transmission tests. However, experiments with artificial transmission have indicated that big vein virus is free in the soil and it is probable that the results obtained by heating the soil at various temperatures apply to the thermal inactivation point of the virus. Since it is possible to kill all of the nematodes in big-vein-infested soil by a moderate amount of heating, it was the writer's original intention to determine

if the temperature at which nematodes were killed coincided with the temperatures required to make big vein soil non-infective. Preliminary tests indicated that the nematodes present in the soil were killed by heating the soil at a temperature of 45° C. for one-half hour. A few nematodes survived a temperature of 40° C. for one-half hour.

Ten 30-gram samples of big-vein-infested soil containing nematodes were exposed for one-half hour at each of the following temperatures: 35, 40, 45, 50, 55, 60, 65, 70, and 75° C. Each 30-gram sample was placed in a test tube with enough water to wet the soil thoroughly. The tubes containing the soil were then suspended in a water bath heated to the appropriate temperature. After the samples of soil had been heat-treated, the soil was removed from the test tubes and added as inoculum to four-inch pots containing soil that had been fumigated with chlorpicrin at the rate of one ml. per gal. of soil. Ten 30-gram samples of the same lot of soil that had received no heat treatment were placed as inoculum in each of ten pots containing fumigated soil to check on the presence of big vein virus in the soil used in the heat treatment tests. Lettuce seedlings grown in chlorpicrin-fumigated soil were transplanted into the pots containing the inoculated soil. The number of lettuce plants developing symptoms of the ten inoculated with soil heated at various temperatures was: No heat treatment, 9; 35° C., 8; 40° C., 10; 45° C., 7; 50° C., 8; 55° C., 1; 60° C., 1. Big vein did not develop where the soil had been heated above 60° C. As indicated previously, it is extremely doubtful that nematodes could play a part in the transmission of the virus since they are all killed at temperatures which did not inactivate the virus.

In addition to the heat treatment of big-vein-infested soil, 100 lettuce seedlings were inoculated with heat-treated juice from the roots of big vein plants. Prior to inoculation the juice was diluted with an equal volume of water. Ten-ml. portions of this mixture were exposed for one-half hour to temperatures ranging from 30° to 75° C. A similar quantity of the same diluted juice was not exposed to heating and was used to check the infectivity of the extracted juice. Ten lettuce seedlings growing in chlorpicrin-fumigated soil were inoculated with the heat-treated juice, five by leaf-rubbing, and five by pouring the juice on the surface of the soil in which the seedlings were growing. The results obtained in this experiment were very similar to those obtained by heating the virus-infested soil. Complete inactivation of the virus occurred at 65° C. The number of plants developing symptoms as the result of pouring inoculum on the soil was: No heat treatment, 4; 30° C., 5; 35° C., 3; 40° C., 5; 45° C., 4; 50° C., 0; 55° C., 1; 60° C., 1. The number of plants developing big vein symptoms as the result of leaf-rubbing with the inoculum was: No heat treatment, 0; 30° C., 1; 35° C., 2; 40° C., 0; 45° C., 0; 50° C., 0; 55° C., 1; 60° C., 0. It has been demonstrated previously that infection does not result from leaf inoculation but takes place through the roots as the result of soil contamination. This

probably accounts for the low percentage of infection obtained in the leaf-rubbing tests in this experiment.

INFECTIVITY OF DILUTED ROOT JUICE

Infective root juice diluted with various amounts of distilled water was added to chlorpicrin-fumigated soil in which lettuce seedlings were growing. Two ml. of diluted juice was poured on the surface of the soil in each pot. Ten plants were inoculated at each dilution. The dilutions tested ranged from equal parts of juice and water to one part of juice to 200,000 parts of water. The number of plants developing big vein of the 10 inoculated at each dilution was: 1–1, 9; 1–100, 8; 1–1000, 6; 1–10,000, 6; 1–20,000, 7; 1–50,000, 6; 1–100,000, 4; 1–200,000, 3. The dilution end point of the virus in root juice extracts appears to exceed one to 200,000.

DISCUSSION

The present investigation has shown that infection of lettuce plants with big vein virus can take place in the absence of a vector. The virus appears to be free in the soil or possibly absorbed on soil particles. Infection appears to occur as the result of root contact with soil containing the virus. The presence of some sort of wounds is probably a prerequisite for infection, but deliberate wounding of the roots does not appear to be necessary. It is probable that infection can occur through natural wounds such as those caused by abrasion during root growth or the breaking of small roots as the result of alternate wetting and drying of the soil. Symptoms of big vein infection appear to develop more rapidly in plants transplanted into virus-infested soil than in seedling lettuce plants grown from seed in infested soil. This is probably an indication that the wounds produced on the roots of lettuce seedlings during transplanting afford a favorable point of entrance for the virus.

The virus of lettuce big vein probably persists in the soil of infested lettuce fields as the result of the continued addition of new virus to the soil from the disintegrating roots of infected lettuce plants. If this is the case, it would explain the observation of Jagger and Chandler (5) that lettuce big vein disease becomes more abundant and greater crop losses result when lettuce is grown year after year in infested fields. The virus can remain infective when diluted by large volumes of water. This is probably an important factor in the persistence of the virus in cultivated fields.

It has been reported (2) that it is possible to transmit big vein virus by artificial inoculation of the foliage of healthy lettuce plants with freshly extracted juice from the roots of big vein infected plants. Results obtained in the present investigation indicated that infection through the foliage of the plant did not occur when infective root juice was rubbed, in the presence of carborundum, on the leaves of healthy lettuce plants. It was found that the symptoms of big vein infection that were obtained by leaf-rubbing with infective root juice developed as the result of soil contamination with the virus contained in the inoculum used to rub the leaves. It is believed prob-

able that the positive results reported by Doolittle and Thompson (2) to have occurred from leaf-rubbing with infective root juice were due to soil contamination. Washing the foliage of artificially inoculated plants immediately after rubbing the infective juice on the leaves apparently carries the inoculum into the soil.

Winter wheat mosaic and winter oat mosaic are caused by soil-borne viruses that are reported to be transmissible by artificial inoculation of the foliage. However, these viruses appear to differ from lettuce big vein virus in that they are present in the foliage of infected plants (6, 13) while in the case of lettuce big vein virus, Rawlins and Tompkins (16), Thompson, Doolittle, and Smith (18), and Pryor (15) have obtained negative results in transmission experiments involving the use of juice extracted from the foliage of infected plants. For this reason it is doubtful that direct comparison of lettuce big vein virus and the viruses causing winter mosaics of wheat and oats can be made. But it should be pointed out that unless special methods are employed to prevent the possibility of soil contamination with the inoculum, the results obtained by leaf inoculation may be misleading if the virus is one that causes symptoms as the result of root infection.

The fact that treating winter-wheat-mosaic-infested soil with a fumigant such as chlorpicrin made the soil non-infective has been cited as evidence of the presence of a vector in mosaic-infested soil (7, 12), the suggestion having been made that the action of the fumigant was probably upon a vector and not on the virus *per se*. Experiments conducted during the present investigation indicate that lettuce big vein virus is inactivated when virus-infested soil is fumigated with either chlorpicrin or dichloropropene-dichloropropane mixture. It is therefore possible that winter wheat mosaic virus can also be inactivated directly by soil fumigation. If this is the case, it is quite possible that a vector is not necessary for the transmission of the viruses causing winter wheat mosaic and winter oat mosaic.

SUMMARY

Negative results were obtained in experiments on nematode transmission of big vein virus with the following species of nematodes: *Aphelenchoides parietinus* (Bastian), *Aphelenchus avenae* Bastian, *Acrobeloides bütschlii* (de Man), *Chiloplacus* sp., *Criconemoides mutabile* Taylor, *Dorylaimus monohystera* Bütschli, *Dorylaimus obscurus* Thorne, *Dorylaimus simplex* Thorne, *Panagrolaimus subelongatus* (Cobb), *Paratylenchus macrophallus* (de Man), *Rhabditis monohystera* Bütschli, and *Tylenchus filiformis* Bütschli.

Transmission of the virus through the foliage of lettuce plants did not take place when the leaves of healthy lettuce seedlings were rubbed, in the presence of carborundum, with freshly extracted root juice of infected lettuce when precautions were taken to prevent soil contamination with the inoculum. Evidence was obtained indicating that infection of lettuce with big vein virus occurs only through the roots. Cases of infection that ap-

peared to indicate transmission by leaf-rubbing were demonstrated to be the result of soil contamination with the infective juice used as inoculum. Big vein symptoms developed in lettuce grown in virus-free soil inoculated with juice extracted from the roots of big vein plants and in virus-free soil inoculated with chopped roots of infected plants. Lettuce plants appeared to have developed big vein symptoms as the result of root contact with soil containing the virus and in the absence of any probable vector or deliberate wounding of the roots.

The virus in the soil or in root juice extracts is inactivated when exposed for one-half hour to a temperature between 60° and 65° C. The virus is also inactivated by fumigation of infested soil with chlorpicrin at 0.23 ml. or dichloropropene-dichloropropane mixture at the rate of 0.46 ml. per gal. of soil. Ethylene dibromide mixture and benzene hexachloride did not inactivate the virus. The virus retains its infectivity when freshly extracted root juice is diluted at least one to 200,000 with water.

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HETERO– AND HOMO–THALLIC TYPES OF DIAPORTHE ON SOYBEANS[1]

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The discovery by Welch (9) that two distinct types of perithecial development of *Diaporthe* occurred on overwintered stems of soybeans raised the question of their identity, since up to this time the only Diaporthe species known on this host was *Diaporthe phaseolorum* var. *sojae* described by Lehman (5). One type (Fig. 1) had perithecia borne singly in an effuse stroma while the other (Fig. 2) had perithecia clustered in a caespitose head. Further, the single perithecial type was heterothallic, the caespitose type, homothallic. The heterothallic type was a weak parasite occurring on maturing plants while the homothallic type was a wound parasite causing cankers and girdling on actively growing hosts. Proof of heterothallism in this genus and the identification of the types are the subject of this paper.

In 1923 Lehman (5) described a species of *Diaporthe* as the cause of the pod and the stem blight of soybean, naming it *Diaporthe sojae*. He reported perithecia only from cultures, but later Wolf and Lehman (10) reported them from overwintered stems. At that time speciation in this genus was based largely on host relationships. In 1933 Wehmeyer (8) in his monograph of the genus, changed the emphasis to morphological considerations and reduced Lehman's species to varietal rank under *D. phaseolorum* (Cke) Ell., thus bringing the soybean fungus into close association with the *Diaporthe* described by Harter (2) on Lima beans. Wehmeyer separated this species from *D. arctii* (Lasch) Nit. on the basis of larger spores, larger perithecia, and shorter ostiolar necks in the latter species. He placed certain forms from Europe on *Medicago* and *Melilotus* in *D. arctii* chiefly on the length of their ostiolar necks. In 1935, Tucker (7) reported *Diaporthe phaseolorum* as the cause of fruit rot of pepper. His cultures were proved homothallic by means of single ascospore isolations. Both alpha and beta spores were present in the cycle. He considered his fungus to be close to the variety of the species recognized by Wehmeyer as var. *batatatis*. More recently Luttrell (6) has reported on the host range of *D. phaseolorum* var. *sojae* and *D. phaseolorum* and showed distinct differences in their pathogenicity toward certain hosts. The variety proved to be a weak parasite and attacked a wide range of plants that had reached a moribund condition. *D. phaseolorum* was greatly restricted. Although Luttrell found both perithecial and nonperithecial strains within the cultures he examined, he did not work with single ascospore isolates.

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MATERIALS AND METHODS

During March, April, and May of 1946 the single and caespitose perithecial types were found repeatedly on overwintered soybean stems collected from the field. Isolations were made from each type by transferring ex-

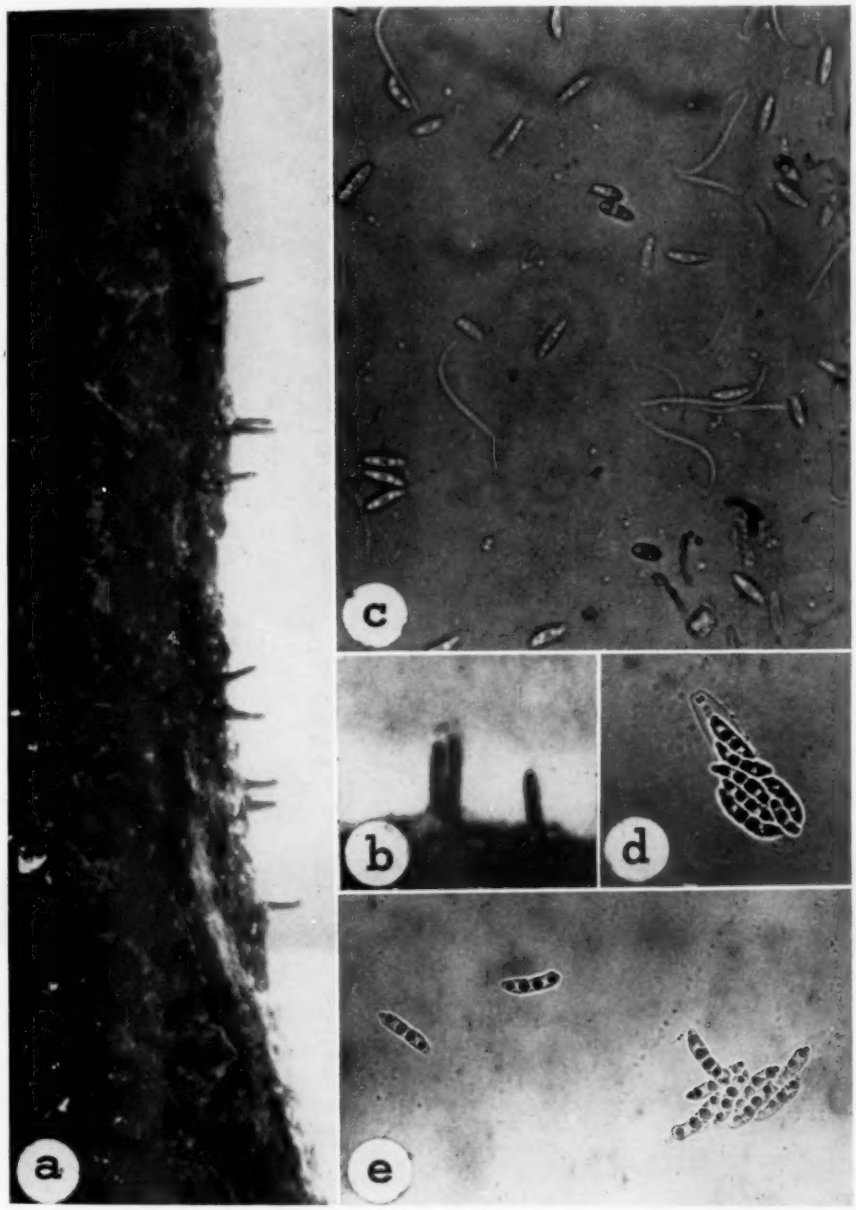

FIG. 1. *Diaporthe phaseolorum* var. *sojae*. a. Perithecia on overwintered soybean cane from the field. b. Perithecial necks showing ascospore discharge on tips. c. Alpha and beta conidia. d. Ascus and ascospores. e. Ascospores.

truded ascospores from perithecial beaks to potato-dextrose agar plates. Cultures and sub-cultures obtained from the single perithecial type produced pycnidia when grown on various agar media and steamed soybean stems

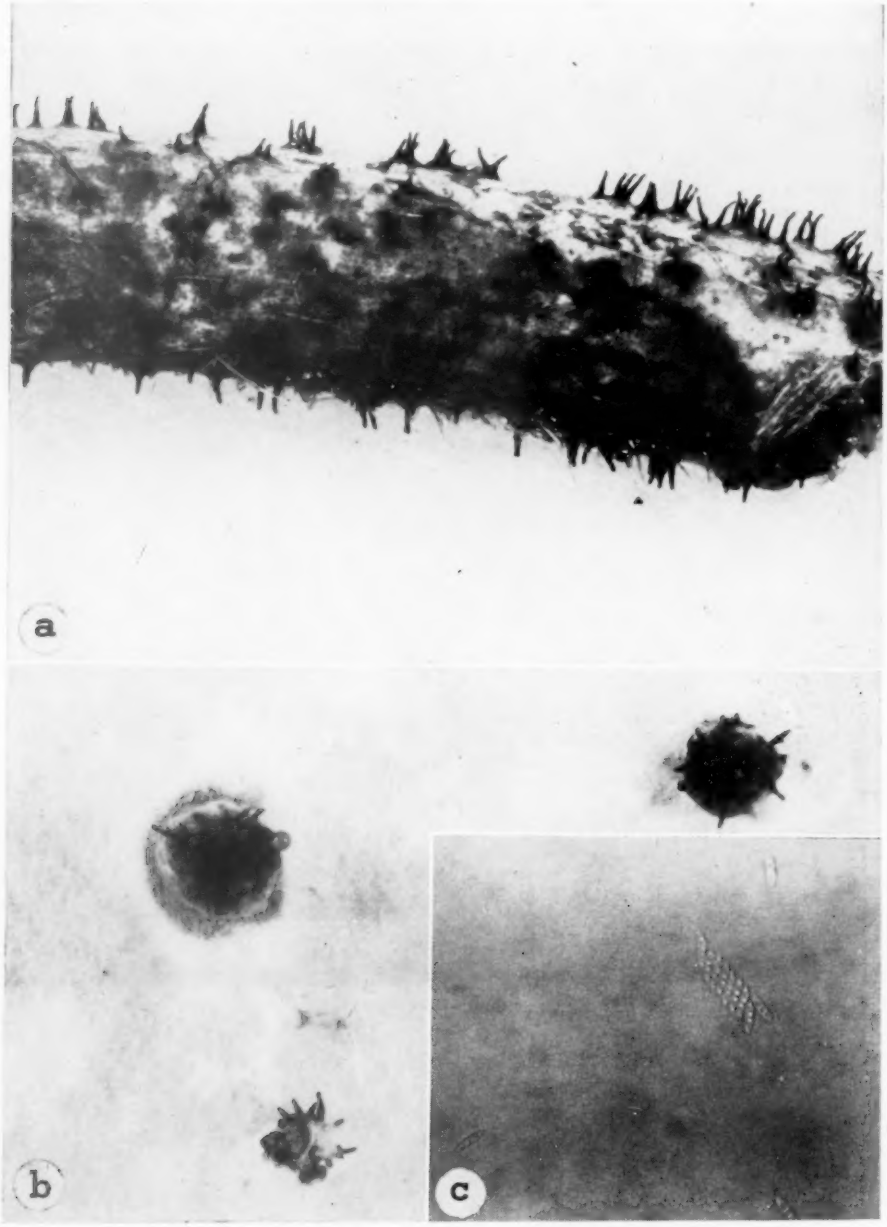

FIG. 2. *Diaporthe phascolorum* var. *batatatis*. a. Perithecia on overwintered cane from the field. Collected at Ames, Iowa, May 24, 1946. b. Clusters of perithecia on potato-dextrose agar plate. c. Ascus and ascospores grown in culture.

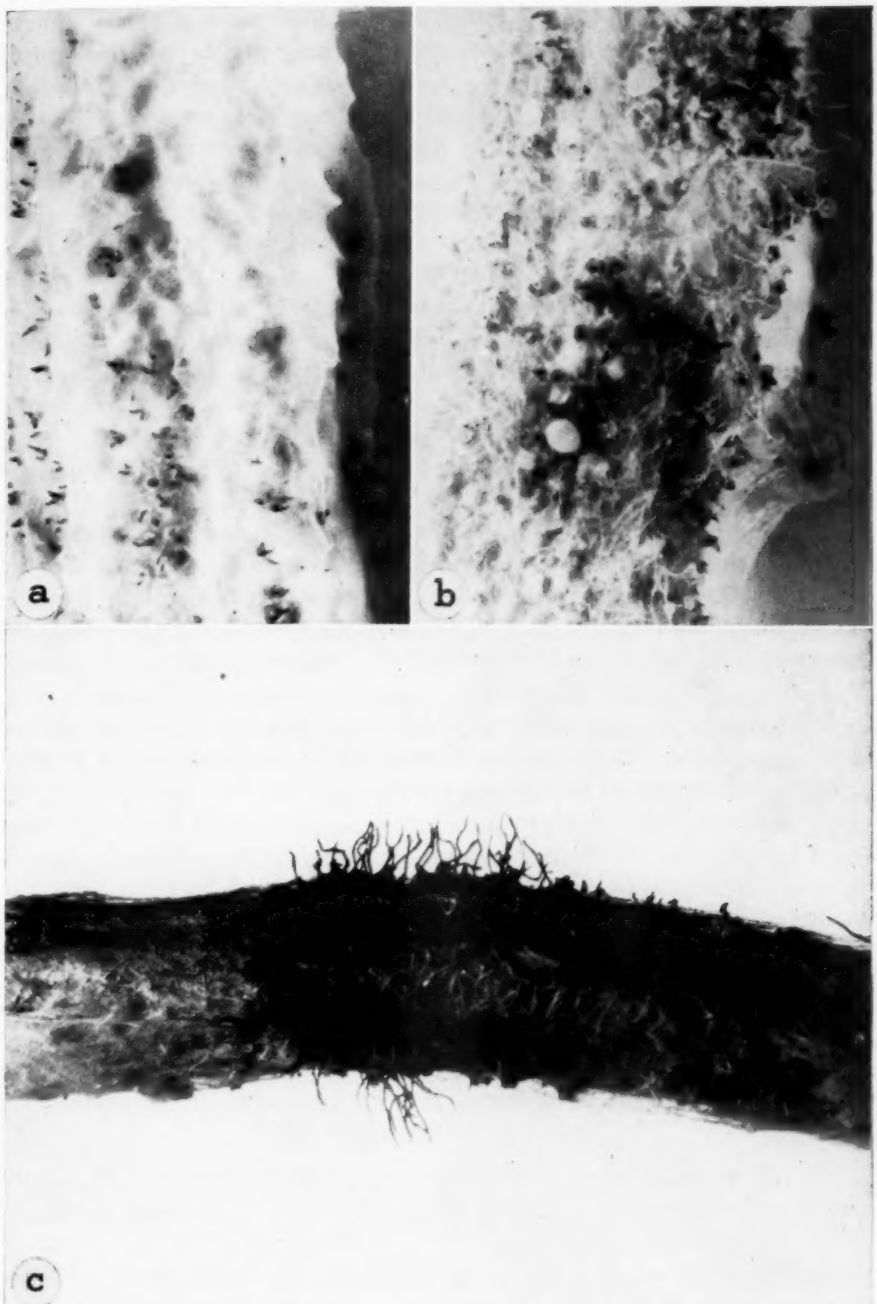

FIG. 3. Cultural characteristics of *Diaporthe phaseolorum* var. *sojae* and *D. phaseolorum* var. *batatatis* on steamed soybean stems. a. Perithecial beaks of *D. phaseolorum* var. *batatatis*. b. Pycnidia and conidial exudate of *D. phaseolorum* var. *sojae*. c. Ring of perithecia at the union of single ascospore cultures 2 and 12 after 35 days.

in the laboratory (Fig. 3, b). Cultures and sub-cultures of the caespitose perithecial type, on the other hand, produced only perithecia when grown under identical conditions (Fig. 3, a). This difference in behavior, observed between the isolates obtained from the two types, indicated that a study of single ascospore cultures of each might prove mycologically profitable.

Single ascospore isolates were obtained by making dilute suspensions of ascospores scraped from the tips of mature perithecia (Fig. 1, b). This ascospore suspension was then poured over 2 per cent plain agar plates. The excess water on the surface of the plate was decanted, leaving only a film over the surface holding the spores. Each plate was incubated at laboratory temperatures for 6 to 10 hours to permit the ascospores to initiate germ tubes. The plates were then placed beneath the microscope where the germinating ascospores were easily observed with the 10× objective.

The single ascospore isolates were made by using a modification of the method described by Lambert (4). A wire loop was made by bending a piece of fine platinum wire around the tip end of a sewing needle, small enough to make the loop fit within the field of the 10× objective when suspended beneath the objective. The wire loop was suspended in such a manner that it would hang immediately above the surface of the agar plate when the 10× objective was in focus with an ascospore resting on the surface of the agar. When the "cookie cutter" was properly attached and adjusted, single ascospore isolates were made quickly and easily. The procedure consisted merely of locating a single, germinating ascospore on the surface of the poured plate and lowering the objective until the wire loop circumscribed a cut around the spore to be isolated. The plate was then transferred to a 30× binocular and the small agar disc lifted out with a dissecting needle. The ascospore was not visible under the binocular but the circular cut made by the wire cookie cutter was clear.

Eighteen single ascospores from 42 trials with the caespitose perithecial type and 12 from 31 trials with the single perithecial type were obtained in pure culture. These were used in all subsequent studies involving single ascospore cultures.

CULTURE DEVELOPMENT OF SINGLE ASCOSPORE ISOLATES OF THE SINGLE AND CAESPITOSE PERITHECIAL TYPES

Successive sub-cultures of the 12 single ascospore isolates of the single perithecial type grown on potato-dextrose agar plates consistently produced nothing but pycnidia. The number of pycnidia produced by the different isolates was variable. In general, mature alpha spores were produced within 2 weeks after the plates were inoculated but spore production continued until the plates became too dry to sustain further growth.

When the 12 single ascospore isolates of the single perithecial type were cultured on steamed soybean stems, pycnidia were again produced by each isolate. The stems were cut into 4-inch sections and placed in test

tubes. The bottom of each test tube contained moist filter paper to prevent rapid drying. This permitted growing the cultures indefinitely if sterile water was added periodically. Such cultures were carried for as long as 70 days but produced only pycnidia. In general, the mature alpha spores were found within 10–14 days after the stems were inoculated and they were produced during the entire period the cultures were grown. Beta spores were produced on sterile stems but they were never observed on agar media.

The 18 single ascospore cultures of the caespitose perithecial type were grown in the same manner and on the same media as were the 12 single ascospore cultures of the single perithecial type. Each of the 18 cultures produced perithecia on agar media as well as on steamed stems. From 5 to 42 perithecia have been observed arising from a single stroma on agar media. When grown on sterile stems 1 to 7 perithecia, generally 2 to 4 occurred in caespitose groups. Pycnidia have never been obtained in either mass or single ascospore cultures originating from the caespitose perithecial type. The fact that it is homothallic is unquestionable. Its failure to produce pycnidia, however, is somewhat confusing.

PRODUCTION OF PERITHECIA BY PAIRED SINGLE ASCOSPORE CULTURES OF THE SINGLE PERITHECIAL TYPES

When repeated attempts to produce perithecia from single ascospore cultures of the single perithecial type failed, it was assumed that the organism might be heterothallic. To test this assumption the 12 single ascospore cultures were paired in all possible combinations. Pairings were accomplished by inoculating tubed, steamed soybean stems with agar-grown inoculum of the two cultures that were to be paired. Two small pieces of agar, one of each of the two cultures being paired, were placed side by side on the steamed stems. Since 12 single ascospore cultures were paired in all possible combinations, a total of 144 pairings resulted. After all pairings were completed the cultures were incubated at laboratory temperatures and periodically checked for pycnidial and perithecial development. Two sets of pairings were made and the results obtained were tabulated (Table 1).

The inoculated stems became covered with a white mycelial growth 3–5 days following inoculation. Within 10–14 days pycnidia were generally produced over the entire surface of the stem. Mature pycnidia always produced the alpha type conidia. The beta type was usually produced simultaneously within the same pycnidial locule with the alpha type. Production of the beta type, however, was irregular and attempts to associate its production with specific cultures or with a specific growth phase failed.

In the first experiment in which all 12 cultures were paired, the first perithecial beaks were observed after 35 days. The beaks were produced singly and were long and black as shown in figure 3, c. The beaks sometimes grew from the base of an old pycnidium or they developed independently and were not associated with pycnidial structures. In general,

they tended to be produced in a collar around the stem between the two pieces of inoculum (Fig. 3, c). When produced at the base of a pycnidium, the perithecial cavity was imbedded in the base of the old pycnidial stroma, developing somewhat beneath and intermixed with the old, empty pycnidial cavities. When perithecia developed in the absence of pycnidial stromata, the beaks penetrated the black, dorsal, epidermal stroma and the perithecial cavities were imbedded in the cortical tissues of the stem, extending down to the vascular tissues. The occurrence of pycnidia and perithecia within the same lesion has never been observed except, as described above, where perithecia developed as a result of pairings.

The inoculated tubes were carried for 75 days before final readings were made and the tubes were discarded. The final reading showed that 33

TABLE 1.—*Perithecial production*[a] *by 12 single ascospore cultures of the single perithecial type when paired in all possible combinations in two separate experiments*

Cul. No.	1	2	3	4	5	6	7	8	9	10	11	12
1	–	+*	*	–	+*	+*	+*	+*	+*	+*	+*	–
2	+*	–	–	*	–	–	–	–	–	–	–	+*
3	*	–	–	*	–	–	–	–	–	–	–	+*
4	–	*	+*	–	+*	*	+*	*	*	+*	+*	–
5	+*	–	–	+*	–	–	–	–	–	–	–	*
6	*	–	–	0	–	–	–	–	–	–	–	+*
7	+*	–	–	0	–	–	–	–	–	–	–	+*
8	+*	–	–	*	–	–	–	–	–	–	–	*
9	+*	–	–	0	–	–	–	–	–	–	–	+*
10	+*	–	–	*	–	–	–	–	–	–	–	*
11	*	–	–	+*	–	–	–	–	–	–	–	+*
12	–	+*	+*	–	+*	0	+*	*	*	+*	+*	–

[a] – Perithecia absent.
+ Perithecia present in first experiment.
* Perithecia present in second experiment.
0 Failures of expected pairings in both experiments.

of the 144 pairings had produced perithecia. The occurrence of these successful pairings are shown in table 1 as +.

Examination of this table shows that if cultures 1, 4 and 12 are accepted as opposites of cultures 2, 3, 5, 6, 7, 8, 9, 10, and 11, the expected successful combinations occurred in all cases except when 1 was paired with 3 and when 4 was paired with 2, 6, 8, and 9. Culture 3, however, did successfully pair with 4 and 12, and cultures 2, 6, 8, and 9 had paired with 1 and 12 in at least one trial.

Since this left 5 pairings which should have produced perithecia the experiment was repeated. In the repetition greater care was taken in setting up the tubes to prevent the inoculated stems from becoming dry. The experiment was carried for 75 days at laboratory temperatures. Final readings were made by examining each inoculated stem under a binocular. Whenever perithecial beaks were observed the observations were confirmed by mounting slides of ascospores under the microscope. The results obtained in this second attempt are shown as * in table 1. In the second

experiment a total of 50 pairings produced perithecia. The reasons why the other 4 pairings (6×4, 7×4, 9×4, and 12×6) were not successful are unknown. They were successful when made in the contrary direction. The successful combinations did, however, confirm the assumptions made from the data in the first experiment. Cultures 1, 4, and 12 reacted alike and 2, 3, 5, 6, 7, 8, 9, 10, and 11 reacted alike. The fact that perithecia were produced when any one culture of the 1, 4, and 12 group was combined with any one culture of the 2, 3, 5, 6, 7, 8, 9, 10, and 11 group was the basis for stating that the single perithecial type was heterothallic. Paired cultures on stem-meal agar produced only pycnidia.

IDENTIFICATION OF THE TYPES

As stated in the review of literature, the earlier treatments of the members of the genus *Diaporthe* were based largely on host relations; and it was not until the monograph of Wehmeyer (8) that morphological grounds replaced those of pathogenicity. At that time Wehmeyer combined those species with elongated ostiolar necks under the name *Diaporthe phaseolorum* and based the species on the form described by Harter (2) from Lima bean. With this form he set up the form causing pod and stem blight of soybean as var. *sojae* and that from sweet potato as var. *batatatis*. The first of these was described by Lehman (5), the second by Harter and Field (3). The latter variety was separated from the former by the fact that it had a valsoid stroma and smaller ascospores.

The heterothallic or single perithecial type of the Iowa material closely resembles the descriptions given by Lehman both in its gross appearance in the field and in culture and in the perithecium, ascus, and ascospore measurements. In both natural and cultural material the perithecia were borne singly on an effuse stroma, had long cylindrical beaks measuring from 347 to 521 μ in length in nature but often exceeding 2 mm. when grown in culture. The perithecia measured 156–260 $\times$ 192–335 μ. They were erumpent through a blackened zone on the surface of the stem with a black line often seen extending from the margin of the stroma into wood and running along the edge of the pith cavity. The asci were sessile, elongate, 8-spored, and measured 37.0–52.0 $\times$ 7.4–12.9 μ (Fig. 1, d). The ascospores were bicellular, slightly constricted at the septum and measured 10.4–18.5 $\times$ 3.7–5.5 μ (Fig. 1, e). Although the ascospore measurements were noticeably larger than the corresponding dimensions given by Lehman, this difference is not believed to be significant; and the authors are inclined to follow Wehmeyer and consider the heterothallic form as *Diaporthe phaseolorum* var. *sojae*. The presence of heterothallism would explain the variations among strains of "perithecial and conidial" forms reported by other workers. Heterothallism hitherto has not been reported for the genus.[2]

[2] Cayley (1) suggested that heterothallism was present in *Diaporthe perniciosa* Marchal but her statement concerned the irregular occurrence of the conidial phases and was not connected directly with the appearance of the perithecial stage and so was not used in the present accepted sense of this word. Wehmeyer (8) also pointed out that Cayley was not dealing with our present concept.

The identity of the homothallic caespitose form is somewhat more difficult to establish. It was distinct from *D. phaseolorum* var. *sojae* in that it actively attacked the stems of the soybean; large light to dark brown lesions circumscribed the stems and the plants wilted and died. Pycnidia have never been found associated with these lesions nor have they appeared in any of the cultures. Single ascospore isolates repeatedly produced perithecia in culture. Perithecia occurred naturally also on overwintered stems in caespitose groups of 1 to 7 per stroma, predominantly in groups of 2 to 4. These perithecia measured 178–325 × 275–390 μ, being more globose (not flattened) than *D. phaseolorum*. In culture the stromata were limited (Fig. 2, b) to circular areas on the surface of the plate. On stem cultures the black line did not extend into the stelar region but was confined to the surface through which the beaks of the perithecia emerged. The beaks were 280 to 546 μ in length and slightly more tapering than the beaks of *D. phaseolorum* (Fig. 1 and 2). The asci were elongate, sessile, with thin evanescent walls and measured 27.2–40.8 × 6.8–8.5 μ. The ascospores were bicellular and measured 8.5–10.2 × 3.4–5.1 μ (Fig. 2, c). The caespitose habit, the globose perithecia, the tapering beaks, the smaller ascospores and the homothallism make it impossible to consider the caespitose type the same organism as described by Harter on Lima bean or by Lehman on soybean. It resembles most closely the strain of *D. phaseolorum* described by Tucker (7) except that it does not produce conidial spores, neither alpha nor beta, such as he figures in his strain on pepper. In this latter respect, it is close to the strains of *D. arctii* reported from Europe on *Melilotus* and *Medicago* (8, p. 7). However, if we accept Wehmeyer's separation on the length of ostiolar beaks, the caespitose organism could not be that species. The authors, therefore, consider it to be a perithecial strain of *Diaporthe phaseolorum* var. *batatatis*, differing from the strains previously described by its lack of a conidial phase, and also in its pathogenicity.

Preliminary infection studies with isolates obtained from both perithecial types have shown that either can infect the soybean stem. Cultures of the caespitose type inoculated into plants growing in the greenhouse have produced the typical symptoms observed in natural infections in the field; lesions circumscribing the stem and causing the plants to wilt and die prematurely. Cultures of the single perithecial type produced infections only on maturing stems. Isolates of the caespitose type are, therefore, considered to be much more pathogenic than isolates of the single perithecial type.

SUMMARY

In the course of investigations of soybean diseases, two members of the genus *Diaporthe* were found which were different relative to pathogenicity and type of perithecial development. One was heterothallic with scattered single perithecia and the other was homothallic with caespitose clusters of perithecia. The former produced typical Phomopsis conidia of the alpha

and beta types; the latter lacked conidial stages. The heterothallic form was recognized as *Diaporthe phaseolorum* var. *sojae;* the homothallic as *Diaporthe phaseolorum* var. *batatatis.* The latter variety actively attacked soybean stems, girdling them and causing the plants to wilt and die. The former variety was less pathogenic, attacking mainly mature plants and producing linear rows of pycnidia on branches and stems.

U. S. DEPARTMENT OF AGRICULTURE

AND

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2,4–D INJURY TO COTTON FROM AIRPLANE DUSTING OF RICE[1]

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During the summer of 1947, considerable damage occurred to cotton in the Gulf Coast area of Texas where nearby rice fields had been dusted with 2,4-D[2] by airplane. Injury to cotton from this source was reported from several coastal counties between Corpus Christi and Beaumont. The greatest damage probably occurred in the Bay City area. A committee of rice and cotton growers found varying degrees of injury on about 10,000 acres of cotton in Matagorda and Wharton counties and they estimated a total loss amounting to 2000 acres. Based on an average yield of one-half bale per acre, losses in both lint and cotton seed were estimated at around $200,000 for these two counties alone.

A warning of the danger to cotton from minute quantities of 2,4-D was sounded by Staten (7, 8) as early as June, 1945. Later, Johnson (5) reported the extreme sensitivity of certain plants to the vapors of 2,4-D that were given off by uncleaned spray equipment. In California, damage to grapevines from 2,4-D was noted by Harvey and Robbins (4) as possibly due to drifting of herbicidal spray. In regard to cotton injury from rice dusting, Brown (2) observed damage in Louisiana, in 1947, and preliminary note has been made (3) of the observations herein reported from Texas. The carrying-over of 2,4-D damage into the seed was noted by Pridham (6) in the case of red kidney bean plants which were sprayed with 2,4-D while the pods were ripening. He found that the seedling offspring showed typical 2,4-D symptoms.

In the areas where these observations were made, rice fields were dusted with 2,4-D during the latter part of May and the first week in June. The cotton had been planted on various dates during March and April. The first injury was noted on the cotton about the tenth of June, at which time the rice growers in most areas discontinued dusting. Regular visits were made throughout the growing season to certain selected fields in order to obtain information as to persistence of the effects of the chemical on the plant and seed. Owing to the variation between fields in regard to such factors as age of plants, fertility, rainfall, and care of the crop, no attempt was made to obtain yield data from damaged fields for comparison with fields that were not affected by the 2,4-D dust.

In some cases, cotton fields showing 2,4-D injury were found at estimated distances of from 15 to 20 miles from the nearest rice fields. However, airplanes with leaky dust-containers may have passed much closer

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Acknowledgment is made to Dr. E. C. Tullis who furnished supplementary information pertinent to these observations.

[2] In this area, the dust mixtures contained about 10 per cent of various salts or esters of 2,4-dichlorophenoxyacetic acid.

than this to some of these fields during or following the dusting of the rice. The fact that cotton fields of 20 acres or more usually showed fairly uniform damage over the entire field would indicate that the dust had drifted for a considerable distance. According to a table by Brooks (1), water droplets about 5 μ in diameter can drift over three miles in falling ten feet when the velocity of the wind is 3 miles per hour. The particle size of the dust used on the rice in this area varied from 4 to 16 μ and the airplanes probably attained heights of from 100 to 200 feet while turning. With wind velocities possibly as high as 10 miles per hour, some of the dust could have drifted, therefore, even farther than the distances mentioned. Since the commercial applications by the airplanes consisted of about 10 lb. of a

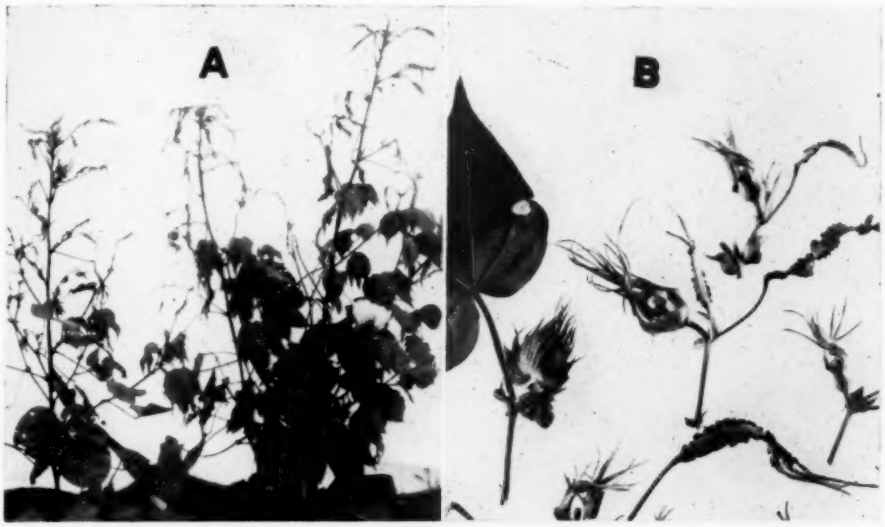

FIG. 1. A. Cotton plants with distorted foliage in a relatively dry field two months after 2,4-D symptoms were first evident. Plants that had set some bolls prior to 2,4-D contact often showed poor recovery even in fields with favorable soil moisture. B. Abnormal cotton squares (flower buds) caused by 2,4-D; normal square at left.

10 per cent dust per acre, the actual 2,4-D dosage on the cotton must have been extremely light in most instances, especially where the cotton was a mile or more from the rice. It is also of interest that one of the weeds (*Caperonia palustris*), for control of which the rice fields were dusted, was often found apparently unharmed among badly injured cotton plants. Occasionally, fields with no 2,4-D symptoms were found in the same vicinity with damaged fields. Protection was apparently provided in some instances by rows of trees on the windward side of the field.

Apparently the cotton plant is extremely sensitive to slight traces of 2,4-D. In spite of such high susceptibility, the plants showed remarkable recovery from severe 2,4-D symptoms. Most of the cotton in this area consisted of Stoneville and Deltapine strains; the latter variety sometimes appeared to be affected less severely than the former by this chemical.

Symptoms of 2,4-D damage, as found in these fields, are shown in figures 1 and 2. The injury consisted entirely in abnormal growth of the foliage, stems, and fruiting structures. No cotton plants were found that were known to be killed by the 2,4-D. The distorted leaves with scalloped margins, narrow lamina, and long tentacle-like teeth persisted on the plants for several weeks, until the middle of August or later. The cotton stems in badly damaged fields were often swollen and the cortex broken open (Fig. 4, A). Frequently spherical, gall-like stem swellings occurred near the ground. Flower buds or squares (Fig. 1, B) and bolls were also ex-

FIG. 2. Cotton leaves showing 2,4-D injury; three injured leaves on left and a normal leaf of the same age on right.

ceedingly malformed. Usually these abnormal fruiting structures were eventually shed from the plant but in some cases elongated or one-sided bolls were produced which frequently had only one or two locules instead of the normal four or five "locks." Although small squares sometimes dried up on recently affected plants, there was no tendency towards excessive shedding of the large squares and young bolls in the fields under observation.

The extent of the original injury seemed to depend to a large degree on the 2,4-D dosage and possibly also on the rate of growth of the plant. The mildest symptoms consisted of a few slightly distorted leaves near the top of the plant. In case of severe injury, all of the new growth was ab-

normal (Fig. 1). Crop losses varied from no apparent loss in slightly affected fields to complete loss in others. Recovery seemed to depend on maturity of the plants, soil moisture, and soil fertility conditions. In fields that received favorable rainfall during the summer, the 2,4-D injured plants made good recovery in most cases. New growth occurred often through the development of lateral branches (Fig. 3) and the original central stalk usually ceased to grow. In some fields, the main stems increased in height and nearly normal bolls were formed among the dis-

FIG. 3. Cotton plant showing recovery from serious early damage from 2,4-D (dead portion of main stem against white background). Normal bolls were formed on strong lateral branches that developed later.

torted leaves which continued to appear for a month or two after the first injury. In most cases, however, especially in plants that were relatively young (prior to first bloom) at time of dusting, strong lateral branches were developed from the lower nodes on the stem and these vegetative branches produced apparently normal leaves, squares, and bolls.

Seed collected from plants that had recovered from initial injury germinated only 40 to 60 per cent. Also, emergence from non-sterile soil was slower with these seed than with seed from normal plants harvested at the same time. In the case of seed from bolls that developed among injured foliage, the tips of the hypocotyls after emergence from the seed coat showed swellings close to the root tip, as shown in figure 4, B. It

seemed remarkable that about 50 per cent of the seedlings from these seed, which were picked in September from bolls that were formed several weeks after the original damage occurred, should show this 2,4-D symptom. Another lot of these seed that was germinated four months later (January, 1948) still showed the same abnormal root development upon sprouting. The cotyledons were not observed to be noticeably distorted by the 2,4-D. The first (and sometimes the second) true leaf was often deformed or filiform in shape and the seedlings were slower than the normal ones in

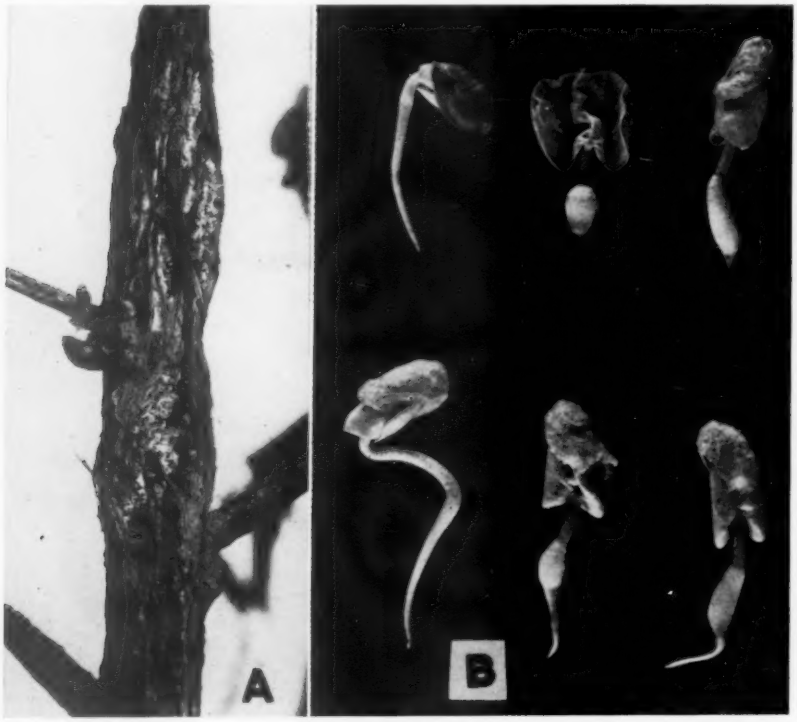

FIG. 4. A. Swelling of cotton stem and cracking of bark due to 2,4-D injury. B. Cotton seedlings with swollen root tips from seed produced by 2,4-D-affected plants. Such seedlings were delayed in emergence and early growth was abnormal. Two normal seedlings are shown on the left.

their early growth. Seedlings from plants experimentally sprayed with 2,4-D required a month or more for emergence, apparently because of inhibition of root development. They outgrew the injury eventually, however, and made normal growth after producing a few distorted leaves.

Continued care and cultivation of cotton fields in the usual manner would seem to be the best procedure to follow in case of 2,4-D damage. This would be particularly advisable in cases of slight damage or where the plants had not reached the flowering stage at time of 2,4-D contact. Retarding of growth was one of the most serious consequences of the 2,4-D injury, especially in the case of relatively mature plants that had a crop

of bolls set when the injury occurred. Continued dry weather further retarded the growth of cotton in some fields. Boll weevils and boll worms become more numerous in most cotton growing areas as the season advances. For this reason, the delayed fruiting of cotton plants that have been damaged by 2,4-D may produce a light crop due to increased insect damage to the new squares and bolls. Therefore, more-than-usual attention to insect control is necessary. Owing to the danger of poor germination and seedling injury, seed from cotton plants that have been affected with 2,4-D should not be saved for planting purposes.

Prevention of damage to susceptible plants or crops growing in nearby fields would necessitate application of 2,4-D weed killers by spraying at low pressures (less than 100 lb. per sq. in.) with nozzles that do not produce a mist. It would also appear best to make the application when the wind velocity is not greater than 3 to 5 miles per hour. Application of 2,4-D by airplane should be made at as low an altitude as possible and care should be taken to avoid high-altitude turning or traveling over susceptible crops in passing to and from the loading point. All airplane spraying equipment should be provided with positive shut-off devices near the nozzle to prevent dripping or leakage. Relatively non-volatile forms of 2,4-D, such as the sodium or triethanolamine salts, should be used whenever possible. Until more is known concerning the dangers and limitations in the use of 2,4-D for killing weeds, extreme carefulness and caution should be maintained at all times. Further costly error in the premature and improper use of the chemical may result in unfortunate delay in the development of these highly-useful hormone-type weed killers.

SUMMARY

1. Symptoms of 2,4-D injury on cotton, extent of damage and recovery, and effects on cotton seed were observed in Texas Gulf Coast fields in 1947, following dusting of rice fields by airplane.

2. Partial recovery of cotton plants from 2,4-D injury, as shown by the production of normal foliage and fruiting structures, occurred in most fields where favorable conditions for growth prevailed during the remainder of the season.

3. In some cases, seed from plants affected by 2,4-D showed poor germination and produced seedlings with swollen root tips (hypocotyls) together with other abnormalities.

4. Possible steps are suggested for the prevention of 2,4-D damage to susceptible crops in nearby fields.

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THE EFFECTS OF MEALYBUGS FEEDING ON PINEAPPLE PLANTS GROWN IN FINELY ATOMIZED NUTRIENT SOLUTIONS[1]

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This project was initiated in order to determine the sequence of symptoms of mealybug wilt. It was known that when plants showed wilt symptoms in the field, the roots were collapsed, but whether this root collapse was primary could not be determined under field conditions with any degree of certainty. A test to study the symptom sequence on pineapple plants grown in both sand and water culture met with similar difficulty. It was not until the method of growing the plants in finely atomized nutrient solutions (2) was devised that it became possible to follow with a considerable degree of accurate measurement, the whole symptom sequence following the infestation of plants with mealybugs.

PROCEDURE

Plants were grown in what has been described as a water-vapor box. This is a simple wooden cabinet with holes drilled in the top into which planting material pieces are fit. Nutrition is provided by means of a finely atomized spray of nutrient solution which circulates continually around the base of the plant. Growth, both of roots and aerial portions of the plant, is very rapid under these conditions, and both roots and leaves can be examined with ease at any time (Fig. 1).

After two pilot runs to establish the technique, the first detailed experiment was set out. Slips from a section of a field free of mealybugs were cured and set in three cabinets holding eighteen plants each. Planting pieces were weighed prior to setting and were distributed in such a manner that each box had a uniform slip weight distribution. Three weeks after setting, measurements of five roots of each plant were made, and these were continued each week until the end of the experiment. Six weeks after the plants were set in the cabinets, they were infested by the fruit skin method (4), two of the cabinets being used for infested plants and one as an uninfested check. It should be recalled that this method of infestation usually involves large numbers of mealybugs in all stages. All the infestations were sprayed out after ten days.

EXPERIMENTAL RESULTS

The Sequence of Symptoms

The sequence of symptoms, of primary interest in the first experiment, is shown in table 1. Root symptoms were rather clearly defined, first by

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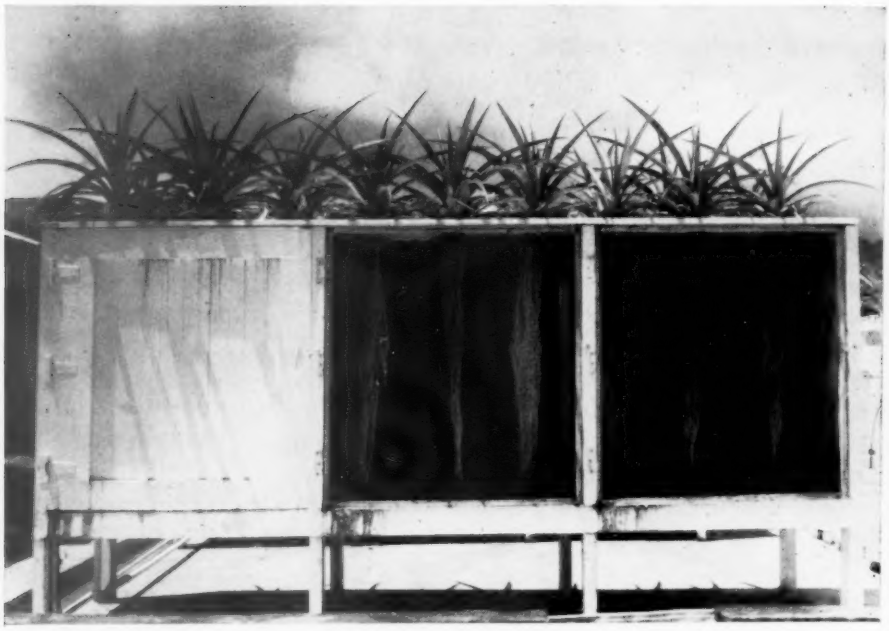


FIG. 1. A general view of the water-vapor cabinet. Two doors were removed to show the root growth.

cessation of elongation and second by a soft collapse of the entire root; the second phase follows closely on the first. Some plants develop leaf symp-

TABLE 1.—*Sequence of symptoms from first indication of cessation of root elongation to appearance of leaf symptoms following infestation with mealybugs*

Plant No.	No. of days after mealybug infestation		
	Root elongation affected	First dead roots	First leaf symptoms
3	42	49	63
3	42	49	63
2A1	42	52	66
4	42	49	54
2	42	58	63
5	49	58	63
3	49	58	63
6	49	49	54
3	49	58	63
4	49	58	63
6	49	49	63
2C2	49	58	63
3	49	49	63
5	56	61	73
1C1	56	58	63
2	56	58	70
2C6	56	76	76
1B1	62	76	70[a]
2B1	62	76	76
2	69	76	76
1A1	72	79	82

[a] 1 median leaf only affected.

toms without the loss of all the roots. The development of new roots is variable, sometimes occurring during the period when the old roots are collapsing, sometimes only after a considerable delay. Leaf symptoms are typical but a very rapid recovery usually occurs which prevents the development of extreme third and fourth stages as encountered in the field (Figs. 2 and 3).

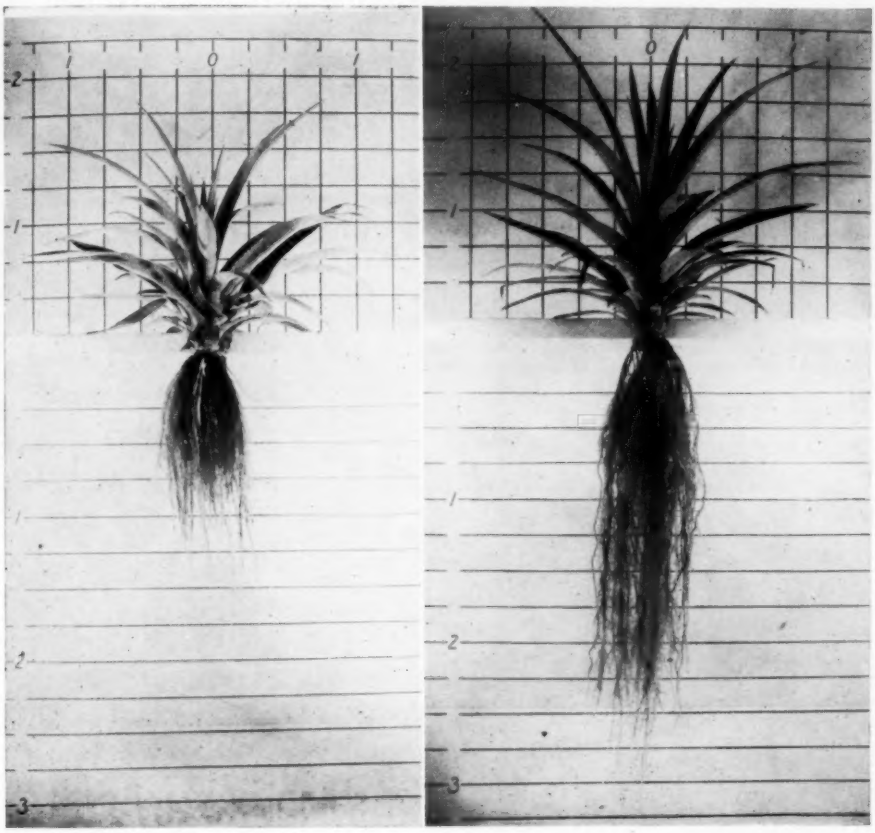
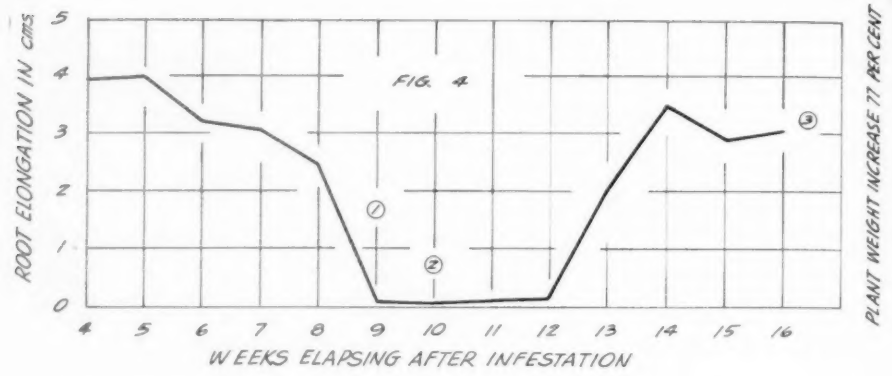
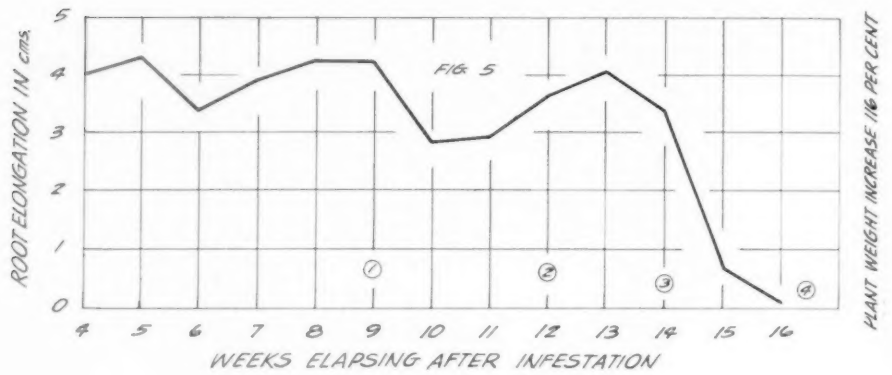


Fig. 2. (Left) Wilted plant with center recovering. Note mass of collapsed roots with short new white roots emerging.

FIG. 3. (Right) Normal uninfested plant.

Table 2 gives some details of root elongation measurements. It should be borne in mind in connection with this table that if a measured root ceased elongating and collapsed, a live root was substituted so that as long as there were live roots on the plant, elongation measurements were continued. The collapse of a root system is progressive. All the roots do not die simultaneously, although once collapse has started, it proceeds rapidly. New roots emerging after the original root mass has died, or during the period of its collapse, soon attain the elongation rate of roots on normal plants.





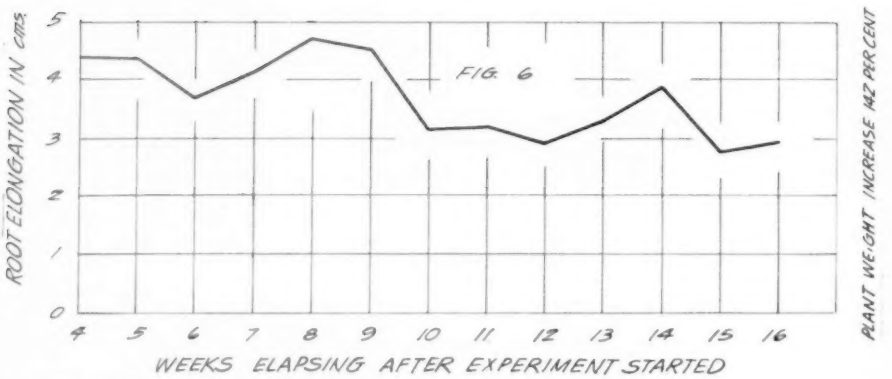


FIG. 4. Plant 1B1 showing typical root collapse with development of new roots.
1. Long roots dead, median leaves reddening on one side.
2. Long roots dead, several white tipped new roots, median leaves reddish.
3. Median leaves wilted, center growing.

FIG. 5. Plant 1A4 showing a case of delayed root collapse.
1. Leaves and roots normal.
2. One median leaf yellow, roots normal.
3. Roots normal.
4. One median leaf yellow distal of feeding area, median whorl with tips wilted. Old roots dead.

FIG. 6. Mean of 18 uninfested check plants.

Table 3 summarizes all the measurements with respect to final symptom class, which for this experiment were as follows; 0 = healthy uninfested plants showing no symptoms, 1 = infested plants showing no root or leaf symptoms, 2 = typical wilt symptoms on plants showing recovery, and 3 = a single case of advanced third stage symptoms. For the infested but symptom-free plants, there is a reduced percentage weight increase and what is probably a significantly reduced length and width of the leaves, with no apparently significant difference in the root number. This was

TABLE 2.—*Root elongation. Summary of weekly means for each plant which showed typical wilt symptoms*

Symptom class[a]	Elongation (in cm.) for week following mealybug infestation							
	4th	5th	6th	7th	8th	9th	10th	11th
3	3.36	2.72	2.74	1.54	0.50	0.06	0.24	0.00
2	3.80	4.28	0.82	0.04	0.04	0.00	3.34	3.88
	3.46	3.12	1.20	0.30	0.16	0.60	1.80	2.12
	4.42	3.34	1.26	1.08	0.94	1.14	1.96	2.56
	3.46	4.64	1.86	0.10	1.36	1.08	1.78	2.74
	4.04	3.90	1.88	0.38	0.14	0.00	2.02	1.62
	3.54	3.98	2.22	0.08	0.10	0.20	1.60	2.18
	4.12	4.62	2.44	0.30	0.28	0.54	2.06	2.36
	4.22	4.68	2.84	0.36	0.68	1.02	2.10	3.46
	4.42	3.66	2.20	0.60	0.66	1.42	3.44	3.90
	4.74	4.58	3.96	0.76	0.10	0.30	0.04	0.00
	3.80	4.16	3.42	1.14	0.08	2.88	1.90	2.40
	2.64	4.90	4.74	3.60	0.60	0.00	0.06	0.72
	4.90	4.54	4.62	3.28	0.80	0.10	0.24	1.94
	3.46	3.54	2.80	2.80	0.98	0.36	0.02	1.70
	3.68	3.98	3.86	3.44	1.72	0.28	0.00	1.54
	4.06	3.30	3.40	4.00	2.84	0.78	0.08	0.00
	4.00	3.94	3.36	2.78	2.72	0.98	0.22	0.08
	4.88	5.24	4.84	4.06	4.40	3.20	0.44	0.48
	4.36	4.44	2.64	3.76	3.86	1.18	0.10	0.10
1[b]	3.93	4.29	3.75	3.85	3.96	3.60	2.88	3.25
0[c]	4.34	4.60	4.15	4.09	4.05	3.73	4.01	3.68

[a] 0 = Healthy uninfested plants showing no symptoms.
1 = Infested plants showing no root or leaf symptoms.
2 = Typical wilt symptoms on plants showing recovery.
3 = A single case of advanced third stage symptoms.
[b] Means of 15 infested but symptom-free plants.
[c] Means of 18 uninfested check plants.

the first indication that a short infestation of mealybugs, insufficient to cause wilt on a particular plant, measurably affected the plant's ability to grow. The increased number of roots in class 2 reflects the development of the flush of new roots with which recovery is associated.

Infestation of Clonal Material in Vapor Boxes and in the Field

In the second experiment, varying numbers of mealybugs were used. Ten-, 50-, and 250-bug lots were applied to each of 12 plants while 18 plants were kept as uninfested checks. On the same date that these plants were infested, a similar series of plants growing in the field were infested

TABLE 3.—*Summary according to symptom class at time of harvesting the plants*

Data recorded	Symptom class[a]			
	0	1	2	3
Number of plants	18	15	20	1
Slip weight (gm.)	208	206	210	194
Final plant weight (gm.)	563	474	386	300
Increase in plant weight (per cent)	170	130	87	55
Final number of roots	61	63	76	61
Total length of live roots (cm.)	2696	2488	475	0
Total length of dead roots (cm.)	24	14	1395	1521
Dry weight of root mass (gm.)	10.8	10.6	5.8	5.1
Mean length of 10 longest leaves (cm.)	49	42	38	36
Mean length of 10 widest leaves (cm.)	4.4	3.9	3.6	3.5
Number of green growing leaves	20	20	19	21
Total number of leaves	55	54	49	42

[a] 0 = Healthy uninfested plants showing no symptoms.
1 = Infested plants showing no root or leaf symptoms.
2 = Typical wilt symptoms on plants showing recovery.
3 = Advanced 3rd stage wilt.

at the same time with mealybugs from the same lot. All infested plants were sprayed out eight and ten days later. Slips were used as planting material and all the slips in both the water vapor boxes and the field plots came from the same clone. In addition to this, plants used in the water vapor boxes were so distributed that slips from individual mother plants were distributed as evenly as possible throughout three water vapor boxes. When the field infestations were made, the series were separated by removing plants to provide a space between each set of infested plants. The numbers of roots on these plants were counted to provide a sample with which the water vapor box plants could be compared with respect to numbers of roots. These data are in table 4, and it will be noticed that the water vapor box plants had a very much larger number of roots than did the field plants.

Only six plants growing in the boxes showed wilt symptoms and in these, five separate mother plants were represented. Five of the plants wilting had been infested with 250 mealybugs each; one with 50 mealybugs.

TABLE 4.—*Experiment 2: Number of roots when plants were infested compared with those on a sample number of field plants*

Number of roots							
Field	Box 1	Box 2	Box 3	Field	Box 1	Box 2	Box 3
3	42	52	71	22	32	53	44
21	73	46	107	4	25	41	73
13	44	67	55	46	66	49	60
32	41	43	62		55	66	46
9	60	55	59		91	94	91
21	25	64	66		77	42	48
6	54	69	56		50	42	37
16	68	54	48		59	52	79
18	87	59	48		63	71	64

Only two of the field plants infested with 250 mealybugs wilted and none following the smaller infestations. This difference between susceptibility in soil and soilless conditions for growth is worthy of note although it has not been consistent throughout these experiments. Soil complexes as effecting susceptibility to wilt have been the writer's concern in previous studies (1, 3, 4). This datum suggests that a factor sometimes present in soils is negative for susceptibility.

The symptom sequences for two of the plants wilted in the water vapor box are shown in figures 4 to 6.

Figure 4 shows the typical symptom sequence in relation to root elongation. There is first a drop in the elongation rate and roots begin to die. All the roots collapse and the typical leaf wilt begins. New roots emerge and the center of the plant resumes growth.

Figure 5 shows a case of delayed interference with root elongation. In this case, one median leaf turned color distal of a heavy concentration of mealybug feeding spots which lay in an almost continuous band about 1 inch wide across the leaf, one month before root failure was apparent. The final wilt symptoms in this case were very mild, extending only to the tips of a median whorl of leaves when the experiment was concluded.

Figure 6 is for the means of the uninfested check plants.

Infestation of Clonal Material for Varying Lengths of Time

The third experiment duplicated experiment 2 with regard to the use of a single clone and the distribution of sister planting pieces from the same mother plant. The number of bugs used was varied however, as well as the length of time they were permitted to feed before the plants were sprayed. Numbers of bugs used were 20, 100, and 500. One set of plants was sprayed out after one week and the second after two weeks. Infestations of field plants were made at the same time with mealybugs from the same sources, and these were sprayed out at the end of the first week. Counts were made of a sample of field plants as described in Experiment 2, with the same result as in that experiment, that is, that the roots of the water vapor boxes were very much greater in number than in the field plants. The experiment was terminated 3 months and one week after the infestations were made.

The use of as many as 500 mealybugs to a plant in a situation open to predators and protected from ants resulted in a general attack by predators so that actually by the end of the second week, the colonies in the unsprayed lot were already greatly reduced. Two weeks after infestation and one week after the first set of plants had been sprayed, an estimate was made of the number of mealybugs on each plant before the second lot were sprayed out. These figures represent a minimum number of bugs on the plant at the time because it is impossible to count all the mealybugs on a pineapple plant unless the plant is dissected. Three sprayed plants originally infested with 500 bugs each, had 1 to 3 bugs visible. The un-

TABLE 5.—*Comparison of wilt incidence in plants grown in field plots and in water vapor boxes. Experiment 3*

No. of mealybugs	Field		Water vapor	
	No. of plants	No. plants wilted	No. of plants	No. plants wilted
20	12	1[a]	12	0
100	12	5	12	1
500	6	5	12	8

[a] 1 median leaf wilted at tip only.

sprayed plants showed colonies of from 15 to 40 in the 500-bug lot, 9 to 23 in the 100-bug lot, and 1 to 2 in the 20-bug lot with 2 out of 6 showing no bugs. Actually there was no difference in the number of plants wilting following the two feeding periods. In the field where infestations were made in an ant occupied area, wilt incidence was greater than in the boxes, as shown in table 5.

In view of the large number of mealybugs used in this test, the reaction of individual plants is of interest. Table 6 permits a comparison between sister plants within the single large clone from which all the plants used were derived. There was no effect on percentage weight increase of plant that could be ascribed to the insect's feeding when wilt did not occur. The susceptibility of sister plants differed because among nine plants wilted, 7 individual mother plants were represented. Further comparisons are

TABLE 6.—*Comparison of weights, in grams, of plants derived from slips of same mother plants. All the mother plants belonged to one large vegetative clone. Experiment 3*

Mother plant No.	Box 4–infested			Box 5–infested			Box 6–check		
	Slip 1			Slip 2			Slip 3		
	Initial slip wt.	Final plant wt.	Inc. (Pct.)	Initial slip wt.	Final plant wt.	Inc. (Pct.)	Initial slip wt.	Final plant wt.	Inc. (Pct.)
A1	320	860	169	380	720	90[a]	340	840	147
A2	420	750	79[a]	440	1340	205	420	1340	219
A3	420	850	102[a]	400	1040	160	380	1040	174
A4	480	810	69[a]	460	1020	122[a]	420	1070	155
A5	420	1200	186	390	800	105[a]	430	1270	195
A6	400	980	145[a]	380	930	145[a]	390	1100	182
B1	540	1400	159	500	1280	156	570	1430	151
B2	410	1260	207	400	1330	233	440	1280	191
B3	410	1190	190	400	1110	178	400	960	140
B4	380	1020	168	400	1050	163	400	1120	180
B5	560	1570	180	520	1460	181	500	1380	176
B6	380	1080	184	370	840	127[a]	380	1100	190
C1	470	950	102	430	1060	147	420	1100	162
C2	400	1130	183	350	1050	200	360	1120	211
C3	380	940	147	370	1040	181	380	1200	216
C4	530	1030	94	530	1190	125	570	1270	123
C5	450	1150	156	440	1310	198	450	1300	189
C6	450	1200	167	420	1250	198	450	1390	209

[a] Plants showing symptoms.

available from table 7, in which the root elongation data and the detailed symptom record are listed. Plants 4A4 and 5A4 were sister plants. One had developed root symptoms early and these were followed by severe wilting. The other did not develop root symptoms until three weeks later and leaf symptoms at the conclusion of the experiment were mild. The

TABLE 7.—*Comparison of root elongation of wilted plants with that of symptom-free plants. Experiment 3*

Plant No.	Mealybugs used	Elongation (in cm.) for week following mealybug infestation									Notes on symptoms
		4th	5th	6th	7th	8th	9th	10th	11th	12th	
4A2	500	3.12	4.40	2.02	0.28	0.12	0.16	0.28	0.20	0.54	Typical severe wilt, 4th stage on median leaves. Center growing.
4A3	500	3.40	4.82	4.32	1.14	0.04	0.12	0.02	0.00	0.00	Do, but not so severely wilted.
4A4	500	2.94	3.70	2.66	1.72	0.18	0.14	0.06	0.30	0.00	As for plant 4A2.
4A6	500	2.92	3.60	4.82	3.30	4.10	2.60	1.70	0.40	0.18	Second stage wilt on one leaf, first stage on others.
5A1	500	3.24	4.88	1.00	0.18	0.16	0.26	0.20	0.28	1.44	Typical third stage wilt. Center growing.
5A4	500	3.58	5.20	4.68	3.30	3.72	2.80	1.54	0.62	0.00	Second stage wilt on two median leaves, first stage on others.
5A5	500	3.34	3.92	4.48	2.60	1.10	0.32	0.08	0.20	0.00	Typical second stage wilt on median leaves.
5A6	500	2.86	4.18	4.08	3.42	3.96	1.62	1.10	0.20	0.00	Second stage wilt on two median leaves, other leaves slightly offcolor.
5B6	100	3.76	4.62	4.80	2.02	0.42	0.06	0.04	0.00	0.00	Typical third stage wilt. Center growing.
4C3	20	4.10	4.48	4.54	3.56	4.32	3.16	2.48	1.56	0.00	No leaf symptoms developed by end of 12th week.
Mn.[a]	500	3.17	4.12	4.34	3.57	3.61	2.59	3.23	2.88	4.73	
Mn.[a]	100	3.37	4.07	4.02	3.00	3.59	2.61	3.01	2.76	3.79	
Mn.[a]	20	3.16	4.04	3.90	3.22	3.50	3.33	3.11	2.70	3.49	
Mn.[b]	0	3.09	4.00	4.01	3.12	3.52	2.59	2.86	2.47	3.17	

[a] Means of symptom-free plants.
[b] Means of check plants.

other pair of sister plants which wilted were 4A6 and 5A6. In both these cases, root symptoms were slow in developing and leaf symptoms were mild. Among the 8 wilted plants infested with 500 mealybugs there is considerable variation in time of onset of root symptoms but a fair agreement between early effect on roots and severity of leaf symptoms. Evidence of root elongation symptoms among the plants infested with 500 bugs, which nevertheless showed no leaf symptoms, is slight; only one of

the four plants in this category, (4A5), showed any root elongation reduction and that for one week only.

Restricted Feeding of Mealybug Strains

In experiment 4 the two clearly defined strains of mealybugs, green spotting and non-green spotting, were compared, using two methods of infestation. In one case the mealybugs were caged on a median leaf as far down as possible. Actually it is not feasible to affix the cages as low down as the insects normally feed. In the second case the mealybugs were applied free to the center of the plant and allowed to settle and move at will.

From 100 to 200 mealybugs of each strain were used in each case. When 200 bugs were caged on a single plant, 2 cages were used, one to each of two leaves, each cage containing 100 mealybugs.

Field plants were also infested at the same time. A comparison of the

TABLE 8.—*Comparison of wilt incidence in plants grown in field plots and in water vapor boxes. Experiment 4*

No. of mealybugs	Strain[a]	Location	Field		Water vapor box	
			No. plants	No. wilted	No. plants	No. wilted
100	G.S.	Free	12	7	6	2
100	N.G.S.	Free	10	3	6	3
100	G.S.	Caged	8	3	8	3
100	N.G.S.	Caged	8	0	10	0
200	G.S.	Free	6	5	6	4
200	N.G.S.	Free	6	0	6	4
200	G.S.	Caged	10	3	10	3
200	N.G.S.	Caged	10	0	8	1

[a] G.S. = Green spotting strain.
N.G.S. = Non-green spotting strain.

relative wilt in the two types of planting is shown in table 8. Although the numbers of plants in each class are too small for final conclusion, it is indicated that free living mealybugs, able to move around the typical area of feeding, are more effective than caged bugs in inducing wilt. In the field the green spotting mealybugs were more effective than the non-green spotting strain.

Effect on root elongation and root collapse was typical for both strains of mealybugs.

Continuous and Intermittent Infestations

In experiment 5 the effect of small colonies of mealybugs was tested, with the manner in which they were applied being the only variable. One set of 18 plants was infested with 10 mealybugs to each plant, three times, at approximately monthly intervals, the second and third infestations being added to the few bugs remaining from the previous colonies. The second set was infested similarly but each infestation was sprayed out after two weeks. There were therefore two weeks' freedom from mealybug feed-

TABLE 9.—*Root growth data from plants infested 3 times with 10 mealybugs. Experiment 5*

Plant No.	Symptom class[c]	Root elongation (in cm.) for week following first mealybug infestation										Root number and length				
		10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	Total No.	No. alive	Length (cm.)	No. dead	Length (cm.)
4A4	2	2.72	2.08	0.24	0.00	0.72	0.50	0.00	0.00	0.00	0.00	76	0	0.0	76	46.8
4C6	2	2.04	2.32	0.34	0.10	0.32	0.12	0.08	0.64	1.10	1.10	65	17	83.9	48	53.8
4A2	2	3.02	4.28	2.66	0.34	0.00	0.10	0.00	0.00	0.00	0.00	69	0	0.0	69	46.8
4C1	2	1.12	2.26	1.60	1.80	0.16	0.54	0.00	0.00	0.38	0.20	61	10	45.5	51	53.1
Mean[a]	0	2.74	3.55	3.12	3.30	2.92	3.05	2.86	2.96	2.44	2.60	65	62	72.2	3	27.0
5A4	2	2.06	1.84	2.30	2.38	2.08	1.04	0.16	0.00	0.08	0.00	89	15	35.6	74	54.8
5A2	1	0.20	1.26	1.18	0.90	1.12	1.54	1.06	0.84	0.62	0.12	55	29	42.6	26	45.2
5B3	1	0.32	0.62	0.76	0.24	0.70	1.26	1.18	1.04	0.20	0.00	60	41	52.3	19	55.7
5B5	1	1.22	0.94	1.04	1.08	1.46	1.84	1.32	1.28	0.00	0.08	47	41	45.8	6	28.9
5C6	1	1.56	1.96	0.76	1.00	1.50	0.50	1.06	1.54	0.52	0.24	49	43	57.7	6	54.9
5C3	1	1.12	1.28	0.98	0.32	1.16	1.86	1.60	2.16	0.70	0.16	54	41	51.3	13	30.3
5A6	1	1.38	1.16	1.92	2.10	2.30	2.70	2.14	2.80	1.24	1.36	87	79	47.3	8	26.8
Mean[a]	0	1.07	0.93	1.22	1.04	1.14	1.49	1.11	1.78	1.36	1.19	65	54	50.7	11	28.9
Mean[b]	0	2.81	3.05	2.72	2.27	2.92	1.97	1.43	1.89	1.50	1.62	88	86	56.7	2	27.3

[a] Means of infested symptom-free plants.
[b] Means of uninfested check plants.
[c] 0 = Symptom-free.
1 = Symptoms limited to a single inner leaf.
2 = Typical wilt.

ing between each separate infestation in the second set of 18 plants. There were 18 check plants.

Each time these plants were infested, mealybugs from the same lot were used to infest plants growing in field plots. Ten plants were infested with 10 mealybugs each and 10 with 100 mealybugs each, and these were sprayed out after 10 to 14 days. The first mealybug infestation was made on November 2; the last on January 3. Root elongation data were taken until March 12 and the final leaf symptoms on April 3.

The first comparison to be made is of the relative wilting in the field plots with the two degrees of infestation and in the water vapor boxes with the two types of infestation. None of the field plants infested with 10 bugs each wilted and of the plants infested with 100 bugs each, 26 per cent wilted. There were 4 cases of wilt in the set of 18 plants which were infested with 10 bugs 3 times without intermittent spraying, and all of these developed to the severe form. Of the 18 plants infested three times but with intermittent spraying, 6 showed wilt symptoms but in only one case did these progress beyond a single leaf.

This is an interesting case of the accumulated effects of mealybug feeding in inducing wilt symptoms on pineapple plants. It is clear that wilt occurred following three successive small infestations of mealybugs none of which alone were capable of inducing wilt symptoms, and that intermittent spraying reduced the extent to which symptoms developed. The length of time necessary for the expression of symptoms, both on the roots and on the leaves was much delayed in comparison with the typical sequence as shown in figure 4. In table 9, the data for the two series are presented for each individual wilted plant. This is for the purpose of calling attention to differences between individuals, particularly between those plants whose symptom expression progressed to complete wilt and those with limited symptoms only. In order to reduce tabulated matter, the data for the first nine weeks are omitted, there being no indication until later of any effect on root elongation. The three symptom classes for this experiment are 0, symptom-free; 1, with symptoms limited to a single inner leaf, and 2, typical wilt.

Referring to table 9, it will be seen that all five plants in symptom class 2 showed typical root collapse. One plant, 4C6, was in the recovery stage when the experiment was concluded and its new root system was well developed. Another plant in this class, 5A4, had a fair new root system developed with no evidence of recovery when the experiment was concluded. The cessation of root elongation in this class indicated that some were affected after the second infestation of mealybugs, others after the third.

Plants in symptom class 1 which showed only a single leaf wilted, varied considerably in their root elongation sequences. Two of them, plants 5A2 and 5B3, showed evidence of two depressions in root elongation, one in the tenth week following the first infestation and another several weeks later.

In all the plants in this class there was more than half the root mass alive at the end of the experiment. It can also be observed that root elongation generally, including that of the symptom-free infested plants, was depressed in the plants which had been sprayed with oil emulsion three times during the course of the experiment.

SUMMARY

When pineapple plants are grown in water vapor cabinets, it is possible to follow the symptom sequence of mealybug wilt with considerable accuracy. The first measurable symptom is the cessation of root elongation. This is followed by death of the roots which usually but not always involves the entire root mass before wilting of the leaves occurs. The first recovery symptom is the production of new roots and this is followed by the restoration of turgor to the central leaves of the plant which proceed to normal growth.

Sub-wilting symptoms, recognizable only by detailed plant growth measurements, sometimes occur and are expressed as reduction in plant growth.

Tests with planting pieces from the same mother plant revealed differences between sister pieces both in susceptibility to wilt and in subsequent development of symptoms. Tests with differential feeding of green spotting and non-green spotting strains of mealybug revealed that free living mealybugs were more effective than those restricted in their feeding area by caging. This was particularly true of the non-green spotting strain.

Continuous infestations maintained by monthly applications of small numbers of mealybugs resulted in more extreme symptom expression than when similar infestations were sprayed out after two weeks feeding.

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LEAF VARIEGATION IN TUNG

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Leaf variegation in tung (*Aleurites fordi* Hemsl.) was first observed in approximately 75 seedlings in a nursery near Cairo, Georgia, in 1930.

The predominant abnormal color in the older leaves was yellow, but there were three or four shades of yellow, and mixtures of yellow and green. Some leaves appeared normal except for an almost imperceptible spot of yellow, perhaps no more than 2 to 3 mm. wide. In other leaves the yellow coloration was extensive, involving one-half to three-fourths of the leaf area. Within the yellow areas there would often be islands, of various sizes and shapes, of normal green tissue. Uniformity or regularity in pattern of colors, such as confinement to the leaf perimeter or to the central area, were not observed (Fig. 1).

The yellow colors showed only faintly on the under surface of the leaves. The color markings were always present on very young affected leaves, but they showed faintly as a greenish yellow.

This striking tung leaf variegation, when it was first observed, was suspected of being due to some genetic disturbance. The possibility of a virus association was not entirely ruled out, although virus infection of woody seedling plants rarely, if ever, is apparent naturally during the first summer's growth in a nursery.

In November, 1930, tung seedlings possessing variegated leaves were marked for later use and observation. Apparently healthy seedlings were also marked. Buds taken from variegated-leaf plants were budded by the ring-bud method into the base of 10 normal seedlings. Buds taken from normal-leaf plants were placed in 10 variegated-leaf plants. Young variegated leaves and succulent shoot tissues taken from affected seedlings were crushed with a small amount of water in a mortar. This liquid was then injected with a hypodermic syringe into buds and young portions of 10 healthy seedlings.

All marked and treated seedlings were transferred from the tung nursery near Cairo, Georgia, to the U. S. Pecan Station, Philema, Georgia, in January, 1931, and planted 12 feet apart in semi-nursery formation. The following spring the budded trees were cut back to about 1 inch above the implanted bud. This plot of tung trees was under observation through the following 5 growing seasons.

The final records showed that trees possessing variegated leaves, while growing in the nursery rows in 1930, continued to form variegated leaves throughout the period of the experiment. Trees that were normal in the

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beginning continued in their normal status. Buds taken from variegated-leaf trees and placed in normal seedlings produced shoots with variegated leaves. Adventitious shoots that grew from points below the implanted buds formed normal-colored leaves. Buds taken from normal seedlings and implanted in variegated-leaf trees produced shoots bearing normal-colored leaves. And trees injected with sap expressed from leaves and succulent tissues of variegated-leaf seedlings produced only normal green leaves throughout the period of the experiment.

In 1936, twenty of the young variegated-leaf trees growing in semi-nursery formation at Philema, Georgia, blossomed and produced a few nuts as the result of open pollination. These were harvested, nuts from each tree being placed in a separate bag, stored during the winter, and planted the following spring.[3]

FIG. 1. Tung leaf with typical pattern of variegation. About ⅓ natural size.

Two hundred and sixteen seedlings grew; of these, 114, or 52.8 per cent, were of the variegated-leaf type. Some variegated-leaf trees produced seeds forming only green-leaf seedlings. Seeds of other trees produced seedlings with leaves only of the variegated type. On the other hand, seeds from some trees formed both green-leaf seedlings and variegated-leaf seedlings in various proportions.

While the number of seedlings giving rise to variegated leaves in comparison to the total number grown was adequate to demonstrate the transmissibility of the variegation character through the seed, the number of seedlings from individual trees was too small to be of any statistical importance for forecasting the pattern of inheritance. Some trees produced as few as two to five nuts, although seven trees bore 15 to 25 nuts each.

The evidence presented suggests the involvement of an inheritance factor

[3] The writers are indebted to Mr. Max B. Hardy, formerly Pomologist, U. S. Pecan Field Station, Albany, Georgia, for growing the tung seedlings and for making the final observations on their performance relative to variegation.

rather than the presence of an infection agent, such as a virus. The variegated leaf character was not transmitted (1) by injecting expressed sap from affected shoots, leaves, and buds into healthy tree tissues; or (2) through the medium of tissue grafts. Buds taken from green-leaf trees formed only green-leaf shoots when implanted into trees having variegated leaves. Buds taken from variegated-leaf trees produced shoots with variegated leaves and the character of variegation did not migrate to adjacent tissues of the stock.

The inheritance of this tung variegation appears to be genetic in type. If it were cytoplasmic, or maternal, then all seedlings growing from variegated trees should have variegated leaves. This did not occur. Four trees produced only variegated seedlings, 2 produced only green seedlings, and nuts from 14 others gave both types of seedlings.

Normal appearing tung trees have been known to transmit variegation to their progeny. Nuts of the Fairchild variety persistently produce some variegated seedlings. This variety originated from a single-leaf seedling, planted in 1930 in the J. B. Wight Nursery, Cairo, Georgia, where the first specimens of tung variegation were observed that same year. This planting was made with seeds secured in small lots from several sources in south Georgia and north Florida. A portion of the seeds came from single isolated tung trees serving for shade around farm homes. Undoubtedly some of those single trees grew from the earliest lots of tung nuts introduced into the United States from China during the first decade of this century, or from seedlings distributed by the United States Department of Agriculture. Mr. Wight received one seedling in 1908, and seeds from this tree were used in part for planting the nursery in 1930.

We can only speculate as to the source of the tung seeds that produced the variegated-leaf plants. There is a possibility that they all came from a single tree, because, although the variegated-leaf plants were scattered, all were in a restricted section of the nursery.

SUMMARY

Although leaf variegation in tung (*Aleurites fordi* Hemsl.) is very infrequent and not important economically, trees possessing a variable leaf pattern composed of three or four tones of green and yellow occasionally appear.

The earliest observation of this phenomenon was in a tung nursery near Cairo, Georgia, in 1930. It was suspected that the abnormal leaf coloration either was associated with the presence of a virus or that some genetic abnormality was involved.

Among 216 seedlings grown from open-pollinated seed produced by 20 abnormal-leaf trees, 114 or 52.8 per cent, were of the variegated-leaf type.

The evidence presented suggests the involvement of an inheritance factor rather than the presence of a virus.

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THE EFFECT OF PRE-PLANTING IRRIGATION ON PATHOGENICITY OF RHIZOCTONIA SOLANI IN SEEDLING COTTON

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Damping-off or soreshin of cotton caused by *Rhizoctonia solani* Kühn is well known, especially in the irrigated areas of the Southwest where the organism occurs generally, attacking cotton and other crops. While much investigational work with seed treatment has been done in an attempt to control the disease, there has been little offered on cultural control.

Lehman[2] has called attention to the fact that the organism in an infested soil is very virulent soon after inoculum is added, but rather quickly loses this extreme virulence. A commonly suggested explanation for this phenomenon is an increase in population of antagonistic or competitive organisms which reduce the virulence of *Rhizoctonia*.

FIELD EXPERIMENT

A field experiment at State College, New Mexico, yielded as a by-product information which suggested that partial control of the disease is possible. The experiment was the conventional two-factor experiment with nine randomized blocks, two types of seedbeds, flat and double-row bed, combined with two quantities of irrigation water, light and heavy, beginning with the initial irrigation. In order to follow accepted farm practice for the locality, it was necessary to irrigate the double-row bed type of seedbed three weeks prior to planting, and the seed was planted in moist soil. With the flat seedbed, the seed was drilled in the dry soil and moisture supplied by an irrigation after planting.

A severe outbreak of *Rhizoctonia* occurred in some of the plots, one to two weeks after emergence of the seedlings, at which time the plots had been given only the initial irrigations. Counts of post-emergence damping-off were made and a summary of the results are in table 1 with an analysis of variance of the data in table 2. No significant differences were obtained between heavy and light irrigations. However, within each of these groups significant differences were secured. Since the experiment was planned for another purpose, the damping-off counts being merely a by-product, the effects of kind of seedbed and pre-planting versus no pre-planting irrigation were confounded and could not be separated. Since it did not seem reasonable to assume that type of seedbed should affect the pathogenicity of a soil-borne fungus, it was assumed that the differences in the virulence of the organism were due largely to the pre-planting irrigation rather than to seedbed effect.

[1] Associate Agronomist and Assistant in Agronomy, respectively.

[2] Lehman, S. G. Cotton seed dusting in relation to control of seedling infection by *Rhizoctonia* in the soil. Phytopath. **30**: 847–853. 1940.

GREENHOUSE EXPERIMENT

A second experiment was set up in the greenhouse with 13 replicates of 4 treatments in 1-gallon stone jars. Treatments were field soil with and without inoculum added, each combined with pre-planting and no pre-planting irrigation. Field soil was passed through a 4-mm. screen and in the infested series a virulent inoculum was worked into the surface inch of soil. In order to simulate field conditions, the inoculum was prepared by gathering from the field, cotton seedlings which had been killed by

TABLE 1.—*Effect of light and heavy irrigations before planting and at planting time on occurrence of post-emergence damping-off in field experiment*

Treatment	Mean percentage post-emergence damping-off
Light irrigation	
Flat seedbed with no pre-planting irrigation	11.69
Double-row bed with pre-planting irrigation	2.23
Heavy irrigation	
Flat seedbed with no pre-planting irrigation	14.50
Double-row bed with pre-planting irrigation	4.57

Least significant difference at odds of 19: 1 is 7.11.

TABLE 2.—*Analysis of data from field experiment on occurrence of damping-off*

Source of variation	Degrees of freedom	Mean squares
Replicates	8	34.45
Heavy vs. light irrigation	1	59.55
Within heavy irrigation group	1	444.02**
Within light irrigation group	1	402.33*
Error	24	53.44

* Probability value less than 0.05.
** Probability value less than 0.01.

Rhizoctonia, grinding and mixing the air dried plants and adding 2.5 gm. of this inoculum to each pot of soil. Pure cultures of the organism were secured from the dead cotton seedlings.

The experiment was begun June 7, the soil in half the pots being kept at optimum moisture content from that time until planting date, June 30, the other half remaining dry. Nineteen acid-delinted, Ceresan-treated seed of the Acala 1517 variety were planted in each pot. Daily counts on emergence and damping-off were made until the termination of the experiment, July 30, at which time all surviving plants were examined for lesions.

A summary of the results of this experiment is in table 3 with analyses of variance of the results in table 4. Since the data were of the discrete type, analyses were made with the original data expressed both in percentages and after transforming to degrees of an angle. Since both types of analyses yielded the same results, the analysis presented is from the data expressed in percentages. Because a significant interaction was not ob-

TABLE 3.—*Summary of greenhouse experiment on effect of pre-planting irrigation on occurrence of damping-off*

Data	Field soil with no inoculum added			Field soil with inoculum added		
	No pre-planting irrigation	Pre-planting irrigation	Difference	No pre-planting irrigation	Pre-planting irrigation	Difference
Percentage emergence	87.85	81.79	6.06	78.12	72.88	5.24
Percentage post-emergence damping-off	2.43	.48	1.95	40.98	26.28	14.70*
Percentage lesion-free plants surviving	68.13	89.21	21.08*	6.27	15.46	9.19

* Significant differences, P less than 0.05.

tained in any case, the more informative analysis appears to be the between and within group type.

In percentage emergence there was no significant difference between pre-planting versus no pre-planting irrigation. There was a significantly lower percentage emergence from the infested soil than from the soil receiving no inoculum, even though Ceresan-treated seed were used. In the noninfested soil, very little post-emergence damping-off occurred in either the pre-irrigated series or the one not pre-irrigated. In the soil group with inoculum added, however, the pre-planting irrigation treatment significantly lowered the percentage of post-emergence damping-off from 40.98 to 26.28.

In the group receiving no inoculum there was a significant difference in the percentage of lesion-free plants surviving in favor of those grown in soil receiving the pre-planting irrigation. The series in this group receiving no pre-planting irrigation produced a considerable number of lesions even though the mortality was negligible. In the soil with inoculum added lesions were produced on the greater portion of the plants

TABLE 4.—*Analyses of variance of data from greenhouse experiment on occurrence of damping-off*

Sources of variation	Degrees of freedom	Mean Squares		
		Percentage emergence	Percentage post-emergence damping-off	Percentage lesion-free plants surviving
Replicates	12	129.75	313.32	247.15
Soils: Inoculum added vs. no inoculum added	1	1129.95**	13456.39**	60295.74**
Within soil groups:				
Inoculum added	1	177.85	1403.12*	549.24
No inoculum added	1	238.83	24.62	3054.28*
Error	36	130.19	205.77	461.64

* Probability value less than 0.05
** Probability value less than 0.01.

whether receiving a pre-planting irrigation or not. The difference in the amount of disease was also evident in the growth of the plants. The plants on the noninfested soil receiving a pre-planting irrigation were outstanding for size probably because of the high percentage of lesion-free plants.

The experimental results reported herein check with field observations made in the irrigated areas of the Southwest where severe infestations of *Rhizoctonia* have been noted to occur more commonly when seed of susceptible crops are planted in dry soil after such crops as alfalfa and sugar beets grown for seed. In such cases, the inoculating material consists of dry infected plant refuse which produces severe damping-off.

CONCLUSIONS

Experimental results indicate that a partial control of *Rhizoctonia* can be secured by giving the seedbed a pre-planting irrigation and thereby allowing an incubation period of several weeks duration. The results suggest that the pathogen is partially inhibited by antagonistic or competitive organisms when the soil is given a pre-planting irrigation.

In rainfall areas early seedbed preparation when soil moisture is present is suggested for partial control of disease.

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THE COLORIMETRIC DETERMINATION OF 2,3-DICHLORO-1,4-NAPHTHOQUINONE ON SEED

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(Accepted for publication April 10, 1948)

A colorimetric method for determining the dosage of tetrachloro-p-benzoquinone on seed was described (1) in a previous publication. Since another fungicidal member (7) of the quinone series, 2,3-dichloro-1,4-naphthoquinone,[2] has been used extensively for the treatment of spinach and beet seed (4) and has shown distinct promise as a protectant for seed of rice (2), peanuts (8), pepper, tomato, maize (3), sorghum (5), and other crops (3, 6), there is need for a comparable, simple method of determining the dosage of this fungicide. Although the method developed for tetrachloro-p-benzoquinone did not prove directly applicable, a modified colorimetric method of analysis was developed. This procedure and data on its reliability for measuring the dosage of three different grades of dichloronaphthoquinone on several types of seed are presented in this paper.

CHEMICAL REACTIONS

The most convenient method of removing the chemical from seed for analysis is to dissolve it in a selective organic solvent that also provides a suitable reaction medium. Acetone was found to be the most satisfactory. The reactions of dichloronaphthoquinone in this solvent with aqueous sodium hydroxide, trimethylamine, and diethylamine were investigated. Intense colors were produced by all of these reagents but they either formed so slowly or faded so rapidly that they were not entirely suitable for photometric analysis. In the presence of 10 per cent aqueous dimethylamine, however, an intense red color developed immediately and remained stable for at least 15 minutes. On longer standing, it faded to a pale yellow that exhibited green fluorescence upon dilution with water.

The exact structure of the red reaction product is not known. Presumably, an unstable intermediate complex of dimethylamine and acetone with dichloronaphthoquinone is formed. The formation of 2-dimethylamino-3-chloro-1,4-naphthoquinone would normally be anticipated since this material is obtained in good yield when the reaction is carried out at high concentrations. Although these two materials exhibit maximum absorption (Fig. 1) in the visible range at 495 mμ, the specific extinction coefficient of the red complex, calculated on the weight of the dichloronaphthoquinone, is more than twice that of the dimethylamino derivative.

Regardless of the chemical nature of the red compound, it was found to

[1] The authors are indebted to H. P. C. Burrell and Geo. E. O'Brien for technical assistance in conducting these experiments.

[2] Sold commercially under the trade-name of Phygon and tested extensively since 1942 under the code U. S. Rubber Compound 604.

be well suited for the colorimetric determination of dichloronaphthoquinone. The color formed quickly, was sufficiently stable for routine analysis and was proportional in intensity to the concentration of fungicide in solution.

MATERIALS AND METHODS

In order to standardize the available equipment and determine the analytical constant for pure dichloronaphthoquinone, tests were made in a

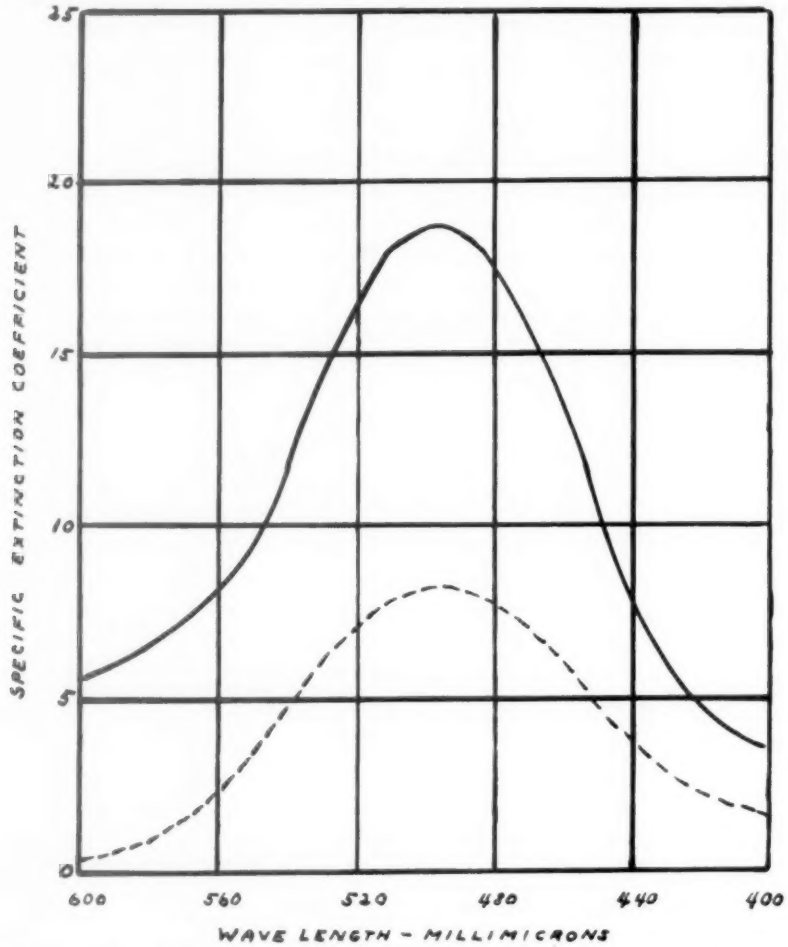

FIG. 1. Absorption spectra of the reaction product of dichloronaphthoquinone with dimethylamine and acetone (solid line) and of 2-dimethylamino-3-chloro-1,4-naphthoquinone in acetone (broken line).

Lumetron colorimeter with several concentrations of a purified sample (m.p. 193° C.) recrystallized from acetic acid. An acetone solution containing 0.25 gm. per liter was prepared and aliquots of 25, 20, 10, 5, and 2 ml. each were transferred to 50-ml. volumetric flasks containing 10 ml. of 10 per cent aqueous dimethylamine and sufficient acetone to make a total volume of ap-

proximately 45 ml. after adding the aliquot. The mixtures were made to volume with acetone and transferred to a 1-cm. absorption cell for photometric analyses in a colorimeter equipped with a green filter.

When the data on the percentage of light transmitted (T) by the various samples were converted to optical densities and plotted against the concentration of dichloronaphthoquinone (grams per liter in the final test solution), a linear relationship was found. The apparent specific extinction coefficient, calculated as described elsewhere (1) was found to be 14.6 with the equipment used. Repetition of the test showed that the results were reproducible to within $\pm$ 1.10 per cent of the total chemical provided the above procedure was followed. Changes in the order of adding the reagents produced small, but significant, differences in the intensity of the color.

For the analysis of seed, 5-gram samples were weighed to the nearest 0.01 gm., washed in three or four portions of acetone (analytical reagent grade), and the combined extracts made up to 50 ml. After filtration through a fluted No. 5 Whatman paper to remove foreign material, an aliquot of 25, 10, 5, or 2 ml. depending upon the dosage anticipated, was transferred to a 50-ml. volumetric flask containing a dimethylamine-acetone mixture and treated as described above. The percentage of dichloronaphthoquinone on the seed was calculated as follows:

$$\text{Percentage of dichloronaphthoquinone} = \frac{5\ \text{Vo}\left(\log\frac{100}{\text{T}}\right)}{\text{KVW}}$$

where Vo is the initial volume of the extract, V the volume of the aliquot, K the apparent specific extinction coefficient, and W the sample weight.

In order to measure the reliability of this method, seed of hybrid dent corn and peas of the variety Perfection were treated with three different formulations of dichloronaphthoquinone (Phygon, about 87 per cent active; Phygon-DDT-S1, a wettable grade about 85 per cent active and containing 1 per cent of DDT; and a special mixture containing 50 per cent of active material in whiting). Each composition was added to glass jars containing approximately 200 gm. of seed weighed to the nearest 0.01 gm. Sufficient material was added to obtain a dosage of 0.48, 0.24, 0.12, 0.06, and 0.03 per cent by weight after a nominal loss on the walls of the container. After the seed and chemical had been tumbled for 30 minutes on a vertical turntable at 28 rpm, the seed were reweighed, correction was made for any loss or gain in the weight of the untreated check, and the dosage of chemical was calculated.

Five-gram samples from each treatment were analyzed in triplicate by the above method and the results corrected for the percentage of active ingredients in each formulation. Emergence data were taken on 16 replicates of 25 seeds each from these lots sown in composted greenhouse soil that was known to be severely infested with seed-decaying fungi. The soil was thoroughly moistened and held at 55° F. for seven days after the seed was sown in order to induce severe seed decay.

EXPERIMENTAL RESULTS

The data obtained on corn and pea seed treated at different dosage levels with the three formulations of dichloronaphthoquinone are given in table 1. The analytical data (mean of three determinations) are in very close agreement with the calculated dosages obtained by weighing. The discrepancies ranged from 0 to 12 per cent at the higher dosages and from 0 to 90 per cent at the lower dosages.

The colorimetric method is inherently as accurate at the lower dosages as at the higher ones since the concentration can be adjusted to the optimum

TABLE 1.—*Relationship of dosage on pea and corn seed as determined by weighing and colorimetric analyses to emergence in infested soil*

Treatment applied to seed	Results on corn seed			Results on pea seed		
	Dose by weighing	Dose[a] by analysis	Emergence	Dose by weighing	Dose[a] by analysis	Emergence
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Phygon	0.47	0.488	93.0	0.44	0.418	94.2
	0.26	0.248	90.0	0.21	0.215	93.8
	0.12	0.121	89.8	0.12	0.124	88.8
	0.08	0.064	83.5	0.05	0.056	81.2
	0.02	0.037	79.8	0.04	0.037	83.5
Phygon—DDT	0.44	0.485	87.6	0.41	0.413	93.5
	0.26	0.262	90.5	0.21	0.219	92.5
	0.12	0.158	87.8	0.15	0.159	93.8
	0.06	0.075	87.5	0.07	0.075	83.8
	0.03	0.059	78.5	0.05	0.058	82.2
Phygon—50 per cent	0.46	0.417	85.3	0.42	0.368	89.5
	0.23	0.228	84.0	0.20	0.208	85.8
	0.14	0.127	85.0	0.15	0.149	88.0
	0.06	0.060	78.5	0.07	0.062	74.8
	0.02	0.033	64.3	0.03 –	0.064	63.2
None	0.00	0.000	16.0	0.00	0.000	37.5
	0.00	0.000	18.8	0.00	0.000	37.5
	0.00	0.000	18.0	0.00	0.000	40.5
Minimum Sign. Diff. 5 per cent	.....	.....	5.2	.....	.....	7.1
Minimum Sign. Diff. 1 per cent	.....	.....	6.8	.....	.....	9.3

[a] Corrected to 100 per cent on basis of percentage active ingredients.

by dilution; so these differences at the lower dosages suggest that most of the error is in the weighing method. A statistical analysis of the triplicate colorimetric tests showed a mean range of 4.3 per cent of the total chemical present, and a standard deviation of 2.53 per cent for the individual analyses. Since the standard deviation of the analytical constant on pure chemical in known concentration was 1.10 per cent, the error attributable to sampling and weighing the seed was in the order of 2.28 per cent of the total dichloronaphthoquinone on the seed.

It is obvious that the analytical data are much more reliable than those from either weighing or seedling emergence. The slopes of the dosage-response curves on both crops were not sufficiently steep to be of practical use in measuring small variations in the amount of chemical applied. The

weighing method is inaccurate because it is based on the measurement of small differences (0.01 to 0.04 gm. of chemical) between two large masses (200 gm. of seed). The only analytical data that might be construed as being unreliable are those obtained on peas with 50 per cent Phygon at 0.03 per cent. Both the seedling emergence and dosage by weight indicate a significantly lower dosage than in the preceding treatment. This one discrepancy is minor, however, in view of the other 29 observations.

Less extensive tests were made on seed of Lima beans (var. Fordhook), spinach (var. Virginia Savoy), and beets (var. Asgrow Canner). Seed were treated at recommended rates of 0.25, 0.50, and 1.00 per cent, respectively. By reweighing the seed, the dosages were calculated to be 0.22, 0.60, and 1.05 per cent by weight. The means of triplicate colorimetric determinations for the three crops were 0.216, 0.573, and 1.03 per cent, respectively. In making these tests it was found that a 20-gm. sample of Lima bean seed gave much more reliable results than a 5-gm. sample while 2.5 gm. of either spinach or beets gave accurate records. When making tests on samples of these different sizes the seed were washed in acetone and made to the customary volume of approximately 10 ml. per gram of seed.

SUMMARY AND DISCUSSION

A simple, rapid method of analyzing seed for dosage of 2,3-dichloro-1, 4-naphthoquinone was developed. The chemical is washed from the seed with acetone and treated with 10 per cent aqueous dimethylamine to produce a red color. The intensity of the color is directly proportional to the amount of dichloronaphthoquinine; so, accurate determinations can be made by measuring the amount of light transmitted. The dosage can be calculated from these data or estimated graphically by plotting the calibration points for known concentrations of the chemical on semilogarithmic paper and fitting the best straight line. The concentration of fungicide in the final solution can be determined directly from this graph.

THE AGRICULTURAL CHEMICALS LABORATORY,
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PHYTOPATHOLOGICAL NOTE

Influence of Nutritional Level on the Susceptibility of Tomatoes to Fusarium wilt.[1]—Recently Walker and Foster[2] have reported that nutritional balance and total salt concentration affect severity of, as well as susceptibility to, pathogenesis by *Fusarium lycopersici* Snyder and Hansen. These authors conclude that "disease devolopment in susceptible or intermediate resistant hosts grown at a temperature optimum for wilt, decreased with an increase in nutrient concentration regardless of the degree of virulence of the pathogen used or the age of the plants within the limits of the studies."

To produce tomatoes of maximum susceptibility to Fusarium wilt for chemotherapeutic studies, plants of the susceptible John Baer variety were grown in builders' sand in unglazed pots. A complete nutrient solution of the same composition as that used by Walker and Foster (0.1 H) was added at weekly intervals at the rate of 25 ml. per pot, the balance of the water requirement being supplied as water. Such plants, when inoculated with the virulent strain of *Fusarium* used in the Wisconsin studies,[3] became diseased only very slowly if at all. Quite obviously the nutritional level in our tests was lower than the minimal level examined by Walker and Foster since their nutrient solution was supplied in drip culture and constituted the sole source of water for the plant. Apparently, also, the plant expresses an increased resistance to disease with a decreased nutrient concentration below the minimal level examined by these authors.

To test this hypothesis, John Baer tomatoes were grown in builders' sand in pots in the greenhouse, at a thermostatically controlled temperature of 83° F. (28° C.). The same nutrient solution was used as was employed by Walker and Foster, and applied at concentrations corresponding to their 0.1 H, 1 H, and 10 H levels of nutrition. Such nutrient was applied every other day at the rate of 25 ml. per pot, the remainder of the water requirement being supplied as water. Inasmuch as the nutrient solution in our tests was not the sole source of water, the levels of nutrition are less than those used by Walker and Foster and are here specified as 0.1 X, 1 X, and

TABLE 1.—*Effect of level of nutrition on susceptibility to and severity of Fusarium lycopersici in John Baer tomatoes*

Nutritional level	Number infected (of 20 plants)	Percentage of plants infected	Index of severity
0.1 X	8	40	26
1 X	19	95	75
10 X	1	5	0

[1] Published with the approval of the Director of the Connecticut Agricultural Experiment Station.

[2] Walker, J. C., and R. E. Foster. Plant nutrition in relation to disease development. III. Fusarium wilt of tomato. Amer. Jour. Bot. 33: 259–263. 1946.

[3] We are indebted to Drs. Walker and Foster for supplying us with cultures of the strains of *Fusarium* employed in their investigations.

10 X, respectively. Twenty plants were maintained at each nutritional level for one month before inoculation, and for one month afterwards before readings were taken. Readings of disease were based on (1) the number of plants infected, as judged by the appearance of vascular discoloration and wilted or dying leaves and (2) the severity of disease expression based on indexes of disease as used by the above-mentioned authors. Results are reported in table 1.

Further differences between lots of plants are shown photographically in figure 1.

FIG. 1. Photograph of tomato plants grown on two nutritional levels and inoculated with *Fusarium lycopersici*. The two plants on the left show the severity of disease when plants were maintained at X nutritional level, whereas the two plants on the right indicate the response of plants maintained at the 0.1 X level. Plants maintained at the 10 X level appeared completely healthy and received an index of severity of 0 (See table 1).

There is evidently an optimum nutritional level for infection of tomatoes and severity of disease by *Fusarium lycopersici*. This lies in the region between the minimum for normal growth and the ideal.—E. M. STODDARD and A. E. DIMOND, Connecticut Agricultural Experiment Station, New Haven, Connecticut.

ANNOUNCEMENT

FORTIETH ANNUAL MEETING

The fortieth annual meeting of The American Phytopathological Society will be held at the William Penn Hotel, Pittsburgh, Pennsylvania on December 6, 7, and 8, 1948. The Northeastern Division will meet with the parent Society. The Council will meet on Sunday, December 5, at 9:30 A.M. Accommodations for 375 persons have been reserved by the hotel for our meeting. You are urged to return to the hotel, as soon as possible, the room reservation card which is being mailed to all members.

Arrangements have been made for meeting rooms for the usual sessions of the Society. However, facilities for special group meetings must also be arranged in advance and it will be appreciated if requests for such meetings are forwarded to the Secretary, Curtis May, at the earliest possible date.

In accordance with provisions of the Constitution of the Society, abstracts of papers to be presented must not contain more than 200 words and must be received by the Secretary not later than October 8, 1948. The Constitution directs the Secretary to return, to the author, abstracts submitted after the closing date, which is 60 days prior to the annual meeting.

Anyone interested in tours through industrial plants in the vicinity of Pittsburgh, may write the chairman of the local committee on arrangements, Dr. W. C. Price, University of Pittsburgh. Consideration is being given to arranging such tours for December 9.

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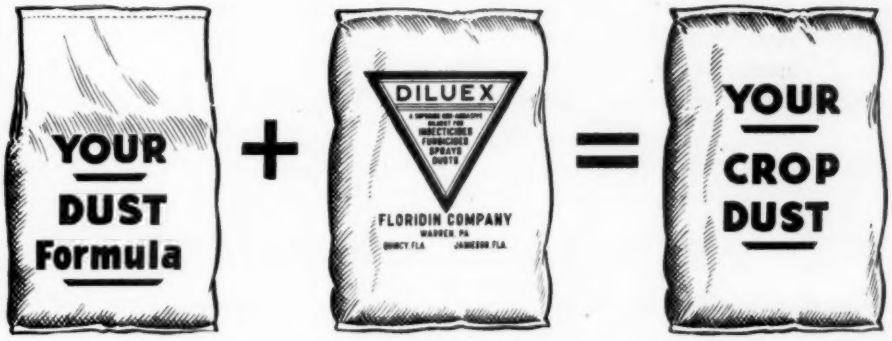

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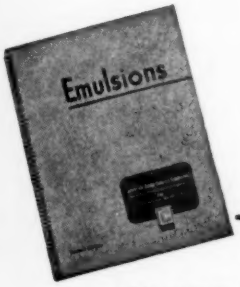

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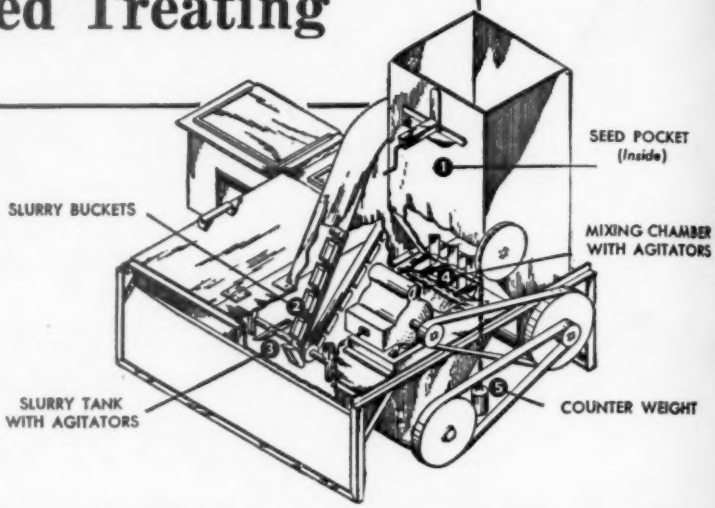

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